Noninvasive assessment of healthy and inflamed skin

Novel perspectives for application in clinical practice with emphasis on rosacea

Jade G.M. Logger

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CONTENTS

CHAPTER 1

General introduction and thesis outline

HEALTHY SKIN

Anatomy

The skin is the largest human organ, has a surface area of approximately 2.0 m^2, and covers the entire surface of our body.[1-3] It serves as the primary barrier against pathogens, chemicals, ultraviolet (UV) light, and mechanical injury. Moreover, the skin regulates body temperature, water release, contains sensory receptors, and has a role in the immune system.[4] It is composed of three layers: the epidermis, dermis, and subcutis (Fig. 1). The epidermis is divided into four layers, from outside to inside: stratum corneum (SC), stratum granulosum, stratum spinosum, and stratum basale. *Keratinocytes*, the main cell type in the epidermis, develop from active stem cells in the stratum basale. Keratinocytes produce keratin and differentiate upwards throughout the epidermis, losing their nuclei in the stratum granulosum, as they move away from nutrients. In the SC, they form a protective overcoat of 15-30 layers of flattened, anucleated keratinocytes (*corneocytes*). The epidermis also contains *melanocytes* which produce melanin to protect against UV light. *Merkel cells* have mechanoreceptors for light touch which interact with cutaneous free nerve endings. Finally, *Langerhans cells* are specialized dendritic cells for antigen presentation and are part of the adaptive immune system.

Below the epidermis lies the dermis, a thick layer of connective tissue (collagen and elastin) giving the skin strength and elasticity. The dermis also contains free nerve endings, blood vessels, and adnexal structures such as hair follicles, hair shafts, sweat ducts, and sebaceous glands. The thin apical layer of the dermis, the papillary dermis, forms finger-like folds extending into the epidermis named papillae. The thick lower layer of the dermis is called the reticular dermis and consists of dense connective tissue. The deepest skin layer, the subcutis, is composed of adipose tissue and plays an important role in thermoregulation, lipid storage, and mechanical shock absorption.

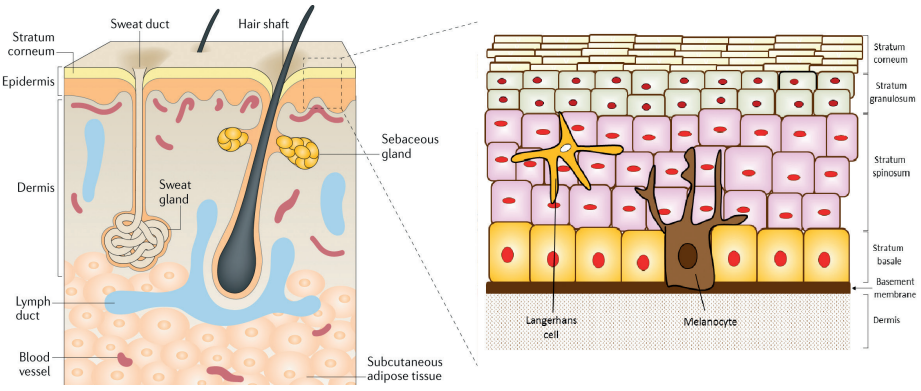

Figure 1. Layers of the skin. *Adapted with permission from Kabashima et al 2018*[5]

Skin barrier function

The main physical/mechanical barrier function of the skin is formed by the SC.[3] It is being continually regenerated, which is important in wound healing.[6,7] The SC prevents excessive evaporative water loss, preventing our body from desiccation.[7,8] Healthy SC contains 10-30% water, important for preserving its elasticity and regulation of desquamation, which takes place at the outermost 3-5 layers.[9-11] The SC has a *brick and mortar* structure (Fig. 2); the *brick* is formed by the flattened, keratin-enriched corneocytes, which are connected by corneodesmosomes.[12] Corneocytes are embedded in the *mortar*, a highly hydrophobic matrix of intercellular lipids, consisting of ceramides (45-50%), cholesterol (25%), cholesterol esters (20%), and free fatty acids (10-15%).[6,10,13] This lipid network is secreted by lamellar granules in the stratum granulosum.[7] The water holding capacity of the SC is mainly formed by these intercellular lipids, the position of the corneocytes, and by natural moisturizing factor (NMF), a highly hygroscopic degradation product of filaggrin inside corneocytes, composed of amino acids, lactic acid, urea, and sugars.[10,14-16]

Various individual and environmental factors are able to induce skin barrier abnormalities, such as filaggrin deficiency, aging, changes in ambient temperature and air humidity, and mechanical stressors disrupting the SC such as traumatic injury or tapestripping.[6,7,9,10] Barrier disruption results in increased transepidermal water loss (TEWL) and reduced epidermal hydration, stimulating production and secretion of cytokines and keratinocyte proliferation, which in turn may cause inflammation.[10,17] Moisturizers can improve skin barrier function by:

- *Occlusion:* providing an exogenous barrier to water loss (although prolonged skin occlusion produces profound negative effects on barrier function, including changed hydration status, barrier permeability, aberrant epidermal lipids, DNA synthesis, changes in microbial flora, and interaction with numerous molecular and cellular processes)[18]
- *Humidification:* attracting and retaining water from the dermis
- *Hydration*: by stimulating synthesis of endogenous water-binding lipids and proteins.[6,7,10,19,20]

Impaired skin barrier function is seen in chronic inflammatory skin disorders like ichthyosis vulgaris,[21] atopic dermatitis,[22-29] and plaque psoriasis.[30-32] Additionally, there are speculations about skin barrier disturbances in acne vulgaris[33], and there is an increasing body of evidence showing barrier impairments in rosacea.[34-37]

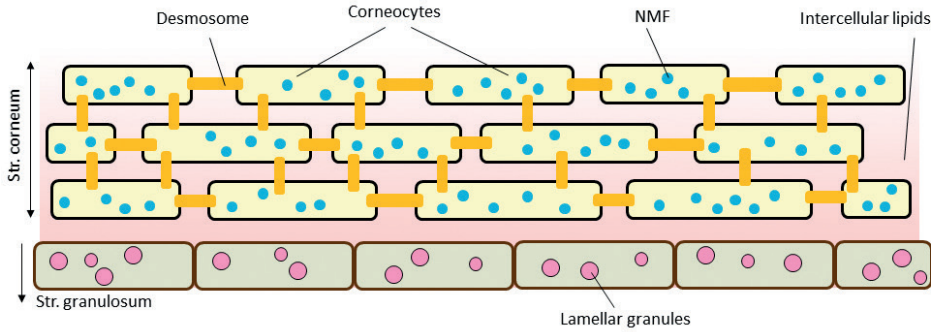

Figure 2. Schematic overview of the skin barrier, formed by the stratum corneum. NMF, natural moisturizing factor

SKIN INFLAMMATION AND INFLAMMATORY SKIN DISEASES

Inflammatory skin diseases (ISDs) involve a broad range of diseases in which inflammation plays an important role.[38] Inflammation is an indispensable factor in the complex defence mechanism of the body against potential harmful endogenous or exogenous stimuli and is involved in tissue repair after damage. Different types of cells in the immune system are involved in skin inflammation. These cells release a variety of substances that can lead to the signs associated with inflammation; *calor* (heat), *rubor* (redness), *dolor* (pain), and *tumor* (swelling). Therefore, it is often challenging to unravel the exact pathogenesis. In this thesis, rosacea, an ISD with facial localization and unclear etiology, serves as a model for the inflamed disease state. Rosacea was chosen because it is localized at the face, a highly visible and cosmetically important region for which noninvasive evaluation is preferred. Furthermore, quantification of its various signs and symptoms is needed. Lastly, it responds effectively to anti-inflammatory treatment. These factors are described below in more detail.

Rosacea

Clinical features and classification

Rosacea is characterized by papules, pustules, erythema and/or telangiectasia at the centrofacial area (cheek, nose, chin, and forehead).[39] The inflammatory and non-inflammatory signs can occur separately or combined in one patient. Symptoms range from very mild to very severe and can cause psychological distress such as low self-esteem, social anxiety, and even depression.[40] Disease course is variable and characterized by exacerbations and remissions. According to the *subtype-based* classification system, which was developed in 2002, there are four rosacea subtypes: erythematotelangiectatic, papulopustular, phymatous and ocular.[39] These are based on defined primary and secondary features in rosacea (Fig. 3). Primary signs are: flushing (i.e. transient erythema), persistent erythema, papules and pustules, and

telangiectasia. Secondary features are: a burning or stinging sensation, plaques, dry appearance, oedema, ocular manifestations, extrafacial localizations, and phymatous changes. Presence of one or more primary features is indicative of rosacea. Secondary symptoms can occur independently or together with the primary symptoms.

It has been previously noted that subtype-based system had some important shortcomings:[42-46]

- There is overlap between subtypes, i.e. signs often span more than one subtype
- Rosacea signs can evolve into other subtypes
- Subtypes do not cover the full range of clinical signs which may confound severity assessment
- It does not recognise certain pathognomonic findings (e.g. phyma).

In 2017, for aforementioned shortcomings, a *phenotype-based* classification system was developed (Table 1) in which one or more diagnostic and/or two or more major features are required for the diagnosis of rosacea. Unfortunately, the phenotype-based classification has not yet been widely adopted due to implementation difficulties. Most studies still apply the subtype-based system.[47]

Figure 3. Clinical symptoms of rosacea. **A,** Centrofacial erythema, papules, pustules, and dry appearance. **B,** Erythema and telangiectasia. **C,** Rhinophyma. **D**, Ocular rosacea. *Reprinted with permission from www.huidziekten.nl (**A-C**), and Schaller et al 2018[41] (**D**)*

Table 1. Phenotype-based classification system of rosacea.[42,48]

Diagnostic features[a]	Major features[b]	Minor features
1. Fixed centrofacial erythema + intermittent intensification 2. Phymatous changes (e.g. nose, ears, chin)	1. Flushing 2. Papules, pustules 3. Telangiectasia 4. Ocular symptoms • Lid margin telangiectasia • Interpalpebral conjunctival injection • Spade-shaped corneal infiltrates • Scleritis, sclerokeratitis	1. Burning, stinging 2. Oedema 3. Dryness 4. Ocular symptoms • Honey crusting • Collarette accumulation at base of lashes • Lid margin irregularities • Evaporative tear dysfunction

[a] These features by themselves are diagnostic of rosacea.
[b] Two or more features may be considered diagnostic.

Epidemiology

Prevalence reports of rosacea vary from less than 1% to more than 20%, due to differences in population, diagnostic criteria, and cultural perceptions of disease.[49,50] Rosacea is mostly diagnosed after the age of 30, and in Caucasians with skin type 1 or 2.[50-52] Frequencies in darker skin types are less reported,[53] possibly due to difficulties in recognizing erythema.[48,54] Gender distribution is reported as equal or female predominant, but phymatous rosacea is most common in males.[50] Ocular rosacea prevalence ranges from 6 to 58%. Rosacea is associated with various systemic diseases such as migraine, depression, inflammatory bowel disease, respiratory diseases, Parkinson's disease and hypertension.[45,48,55] The cause of these associations is yet unknown but it is hypothesized that shared neurogenic, inflammatory, and vascular abnormalities may play a role.

Pathogenesis

The aetiology of rosacea is uncertain, but probably multifactorial.[35-37,45,48,56-59] Factors that may be involved are dysregulation of the innate and adaptive immune system (T_h1/T_h17 cell type inflammation), neurogenic dysregulation, vascular abnormalities, micro-organisms such as *Demodex* mites, skin barrier dysfunction, and genetic factors (Fig. 4).

Demodex mites are obligatory parasites of human sebaceous follicles (Fig. 5). These follicles are mainly present in the face, especially on the nose, cheeks, forehead and chin.[60-64] *Demodex* feed on epidermal cells and sebum. There are two species; *Demodex folliculorum* (length: 0.3 mm), residing grouped in hair follicles, and *Demodex brevis* (length: 0.2 mm), staying solitary in the sebaceous glands. Steadily increasing with age, there is up to a 100% colonization rate of *Demodex* mites in adults.[61,65] Since their discovery, the pathogenicity of these mites has been debated. Their biological function as a component of normal skin microbiome is unknown. Higher mite numbers have

been found in rosacea skin compared to healthy controls, correlating with an increase in inflammation markers, possibly leading to inflammatory skin changes.[49,63,65-71] Proposed mechanisms are mechanical blockage of hair follicles, foreign body reactions, and secretion of waste and bacteria.[60,63]

Besides from causative factors, various triggers have been described that can aggravate rosacea symptoms, such as heat, stress, UV light, spicy food, alcohol, and hot beverages.[49] They are thought to induce rosacea via dilation of cutaneous vessels, after stimulation of transient receptor potential (TRP) channels on sensory neurons and keratinocytes (Fig. 4).[48]

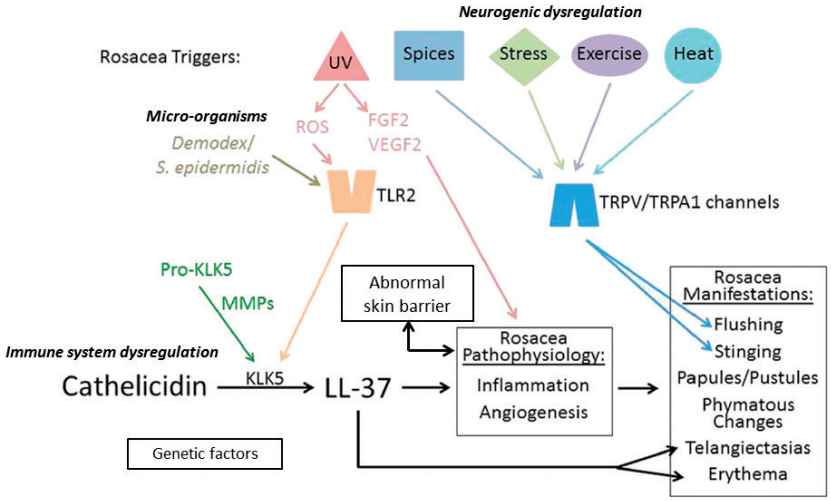

Figure 4. Supposed multifactorial pathogenesis of rosacea. *Adapted with permission from Two et al 2015*[35]

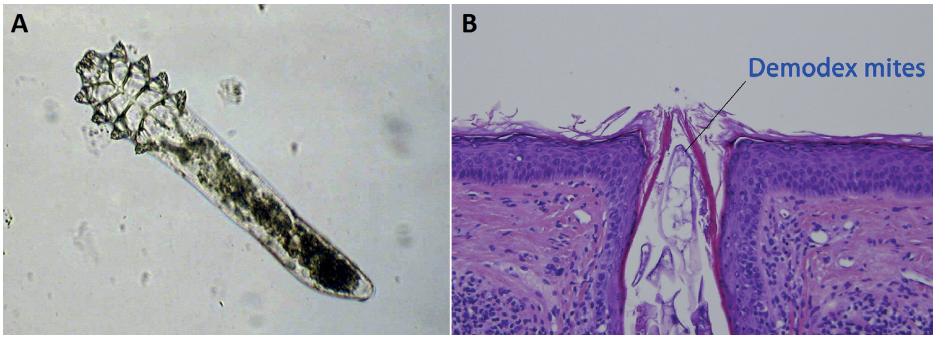

Figure 5. *Demodex* mite. **A,** Under a light microscope. **B,** In a skin biopsy. *Reprinted with permission from Elston et al 2014*[64]

Treatment

Rosacea is treatable but not curable. Explanation about the chronic character of the disease and general skin care should be given to all patients. General skin care topics that should be discussed are adequate sun protection (factor 30+), trigger avoidance, and use of moisturizers and mild cleansers. The available treatment options to reduce or clear the various symptoms are presented in Table 2. Treatment decisions should be based on presenting features and different treatments can be combined to increase efficacy. Papules and pustules respond better to treatment than erythema and flushing, which may be persisting. For moderate inflammatory rosacea with papules and pustules, ivermectin is the most effective topical treatment option, having dual anti-inflammatory and anti-parasitic properties.[72] Oral antimicrobial agents (usually 8-12 weeks) are indicated if response to topical agents is inadequate or in severe inflammatory presentation. If severe cases with inflammatory papules and pustules do not respond to oral antibiotics, or if these symptoms recur after the discontinuation of oral antibiotics, treatment with low-dose oral isotretinoin (usually 12-16 weeks) can be considered. Continuation of topical therapy after remission of clinical symptoms is recommended to reduce the risk of recurrence. Laser therapy may be beneficial in patients with facial erythema or flushing that do not respond to conventional anti-inflammatory therapy. Phymatous and ocular rosacea require separate treatment. For phymatous rosacea, it is desirable to first treat the inflammatory component, before switching to physical treatment modalities. For ocular rosacea, immediate referral to an ophthalmologist is recommended in case of corneal involvement, acute pain or vision loss, or when paediatric ocular rosacea is suspected (which may progress more severely).[45,48,73-77]

Table 2. Treatment options for rosacea.[77]

Erythema/ flushing	Telangiectasia	Papules and pustules		Phyma	Ocular
		Mild/moderate	*Moderate/severe*		
Brimonidine gel Laser therapy (PDL, YAG, IPL) *Inflammatory component: add topical therapy* Ivermectin Metronidazole Azelaic acid	Laser therapy (PDL, YAG, IPL)	*Topical therapy* Ivermectin Metronidazole Azelaic acid *No effect after 8-12 weeks: add oral therapy*[a] Doxycycline Minocycline Azithromycin[b]	*Topical therapy* Ivermectin Metronidazole Azelaic acid *Combined with oral therapy*[c] Doxycycline Minocycline Azithromycin[b] *No effect after 8-12 weeks: stop oral therapy* Isotretinoin (oral)[c]	*Inflammatory component* Retinoid (topical) Doxycycline (oral) Tetracycline (oral) Isotretinoin (oral) *No inflammation* Physical treatment (ablative/electro-surgery, laser therapy)	Lid hygiene Artificial tears Omega-3 fatty acids suppletion *No effect after 2-4 weeks: refer to ophthalmologist* Cyclosporin eyedrops Doxycycline (oral)

[a] Usually given for a period of 8-12 weeks. [b] For pregnant females. [c] Usually given for a period of 12-16 weeks.
PDL, pulsed dye laser; YAG, yttrium aluminium garnet; IPL, intense pulsed light

Diagnosing rosacea: current limitations

Currently, clinical assessment represents the gold standard to diagnose rosacea.[42,44] Various numerical scales exist to score erythema, telangiectasia, papules, pustules, and global impression.[42,78,79] These scales are subjective, not validated, require training, and are therefore prone to interobserver variability, questioning the meaning of reported outcomes. Comparison of individual studies is therefore challenging.[80] Also, solely visual evaluation cannot reveal ongoing sub-surface skin processes, and the human eye is unable to notice subtle changes over time.[39,78,79,81-83] Unfortunately, there are no rosacea-specific biomarkers, and histopathological findings are nonspecific; biopsies are usually only obtained to rule out other diagnoses.[35,45,83-85] Moreover, it is challenging to select an appropriate biopsy sample site due to the heterogeneity of inflammatory areas in rosacea. This can lead to non-specific and descriptive diagnoses. Invasive methods such as skin biopsies are also not preferred in rosacea for cosmetic reasons, and rosacea skin is usually very sensitive and gets irritated easily. Tissue processing takes time, is tedious, operator-dependent and not free from artifacts.[86] Lastly, biopsies can lead to inflammation, scarring, and alteration of examined skin, hindering temporal monitoring of the same skin site.

Summarized, current diagnostic assessment in rosacea has important shortcomings, being:

- The clinical spectrum is wide
- Current clinical scoring scales are subjective
- Clinical scoring does not provide information about subsurface processes
- Facial biopsies are invasive and results are non-specific.

To achieve optimal results, rosacea treatment is preferably adjusted to clinical symptoms and disease severity.[36,87,88] Therefore, standardized, objective, reliable, and preferably noninvasive measurement tools in rosacea are needed. With noninvasive techniques the same facial skin can be easily monitored over time, without causing damage or skin alteration.

NONINVASIVE MEASUREMENT METHODS

A variety of noninvasive diagnostic tools have been developed and investigated in dermatological research,[12,89-92] divided in imaging and biophysical methods. These techniques are also widely used in ISDs such as rosacea, but a comprehensive overview is lacking. Therefore, an overview will be presented in this thesis. The studies in this thesis focus on visualisation of the epidermis and dermis, and on assessment of skin barrier function and erythema. Noninvasive tools that are suitable for these purposes are explained in more detail below.

Reflectance confocal microscopy

Reflectance confocal microscopy (RCM) provides *in vivo* visualisation of the skin at nearly histologic resolution.[93] A commonly used commercially available device is the VivaScope 1500 (Fig. 6).

Figure 6. Vivascope 1500. A metal ring with an adhesive window is applied to the skin with a drop of water in between. The VivaCam is used to obtain a dermatoscopic image. Then, ultrasound gel is applied on the window and the objective lens housing is attached to the magnetic metal ring. After starting the laser, black and white images appear on the computer screen. *Reprinted with permission from the thesis of M. Peppelman*[94]

Technical and practical aspects

The RCM uses a low-energy, near-infrared 830 nm laser light source, which penetrates into the skin and illuminates a horizontal, focal plane within the tissue.[93] Light is back-scattered from certain tissue structures at the focal point, and enters a detector through a pinhole aperture (Fig. 7). Out of focus light from other tissue points are blocked from detection.[95] The lateral (horizontal) resolution of RCM is 0.5-1 µm and the axial (vertical) resolution is 3-5 µm. RCM produces images parallel to the skin surface (*en face*) up to a depth of approximately 250 µm, enabling visualisation of the epidermis, papillary dermis and superficial part of the reticular dermis. Contrast is provided by refractive index differences between cells and surrounding tissue. Images are viewed in greyscale. Melanin and keratin have a high refractive index, appearing bright in RCM. The reflectivity of white blood cells, chromatin, collagen, and elastin is lower, appearing darker (Fig. 8).[93,96-99] Individual layers of normal skin can be distinguished clearly.[93,100,101] However, reflection differences are present depending on anatomical site, skin type, and extent of UV-light damage.[101-103]

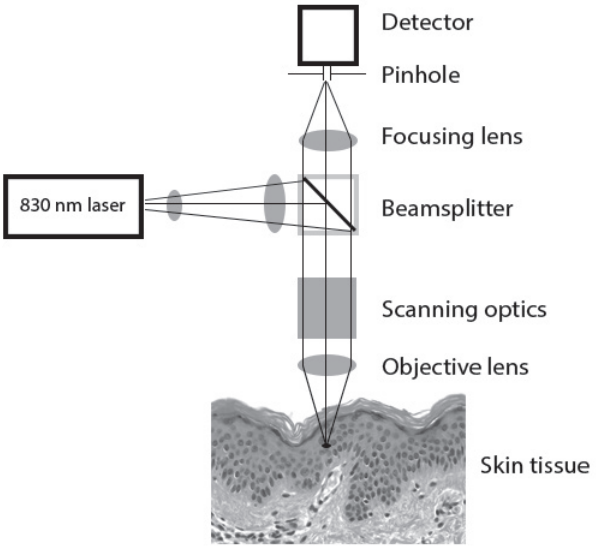

Figure 7. Schematic representation of the principle of the reflectance confocal microscope. *Reprinted with permission from the thesis of M. Peppelman*[94]

A standard RCM image depicts an area of 0.5x0.5 mm^2 of the point of interest in the skin (confocal image) at 30x magnification. Confocal images can be stitched together by horizontal movement of the objective lens into a 2-dimensional mosaic up to 8x8 mm^2 (VivaBlock).[104] Moreover, the objective lens can move vertically to capture images in depth (VivaStack). In addition, movies can be captured to document dynamic processes such as blood flow.

Applications in dermatology

RCM allows comfortable, noninvasive imaging of the skin without causing pain or scarring. Skin structure is not altered due to tissue processing or staining, which is especially advantageous in facial dermatoses. Moreover, an inflammatory response, which may interfere with the diagnosis and study observations, is prevented. RCM allows temporal monitoring of the same skin location for assessing therapy efficacy and enables visualisation of disease progression.

Initially, RCM was mainly used to diagnose melanocytic lesions and (non-)melanoma skin cancer, as melanin and melanosomes are strong contrast sources for RCM imaging.[105-116] Later, RCM application was expanded to evaluate various ISDs and infestations with fungi and parasites.[117-120] RCM has been used in psoriasis,[121-125], acne vulgaris[126-129], and also in rosacea, but only to study *Demodex* mites.[130-135] So far, inflammatory and vascular parameters have not been studied in rosacea with RCM, therefore these will be subject of this thesis.

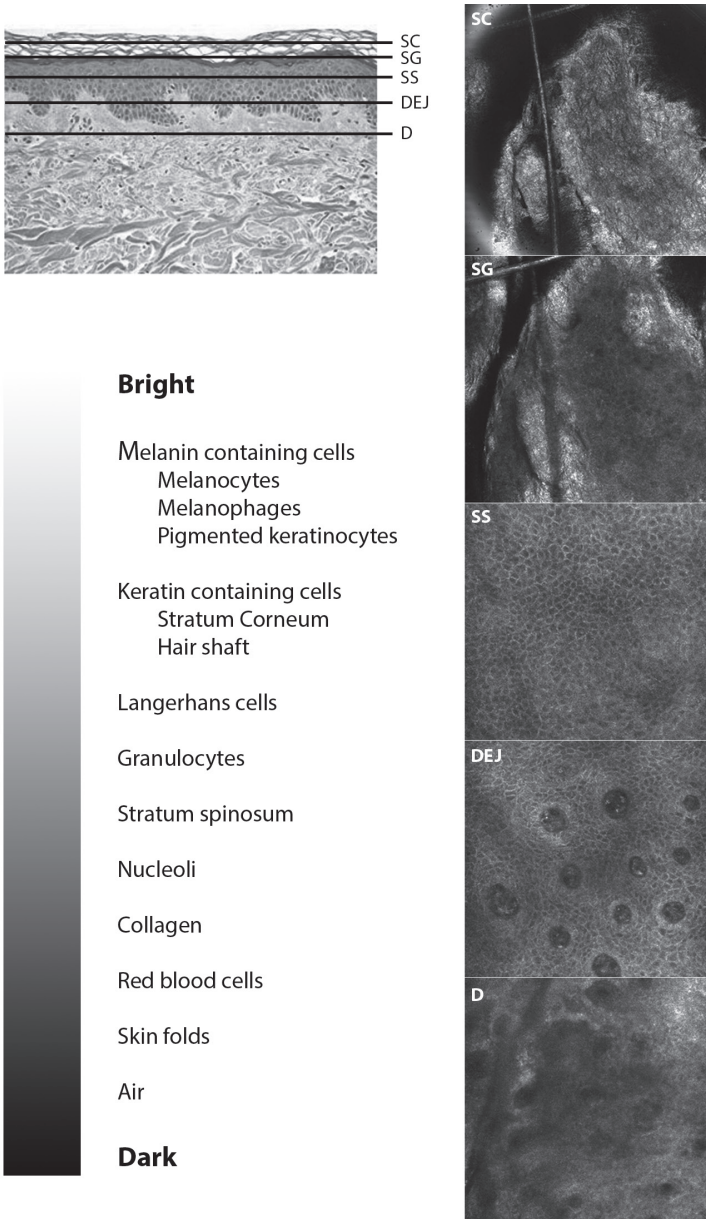

Figure 8. RCM images of the skin and illustration of the refractile structures in decreasing brightness. The transversal histological image shows the levels at which the RCM images are obtained. The stratum corneum (SC) appears as bright grey, anuclear cells. The stratum granulosum (SG) and stratum spinosum (SS) can be recognized by the appearance of dark round nuclei with surrounding bright cytoplasm in a regular honeycombed pattern. At the dermal-epidermal junction (DEJ), the dermal papillae are visible; dark round areas surrounded by a rim of bright basal cells. The dermis (D) will appear as a bright fibre-like network and dark linear blood vessels. *Reprinted with permission from the thesis of M. Peppelman*[94]

Biophysical techniques

A wide variety of biophysical analysis techniques have been developed to investigate and monitor the skin barrier function.[136] These techniques are often hand-held probes placed in contact with the skin, allowing *in vivo* measurements. Tools to study transepidermal water loss (TEWL), SC hydration and erythema are discussed below.

TEWL devices

TEWL corresponds to the flux in water vapor, also including passive sweat gland activity, that moves from inside the body to the external environment by passive diffusion across the SC.[13] TEWL measurements are taken over a fixed area of SC over a fixed period of time, and is expressed as [g/m^2/h].[136] Skin barrier dysfunction results in increased TEWL, and these changes relate well to the degree of impairment.[12,137] Contrary, reduced TEWL is linked to a stronger or recovering skin barrier.[138] Monitoring of the variability in TEWL is therefore useful to evaluate therapy efficacy. TEWL shows significant variation at different anatomical sites due to differences in the nature of the intercellular lipids and/or corneocytes, sweat gland activity, occlusion, skin temperature, skin thickness, and microvasculature.[139-141] Numerous instruments have been developed to measure TEWL, with a major application in cosmetic studies to evaluate the effect of moisturizers on the skin barrier. Three measurement principles are used: open-, unventilated-, and condenser-chamber devices, each with their own advantages and limitations (Fig. 9).[12,13,142,143]

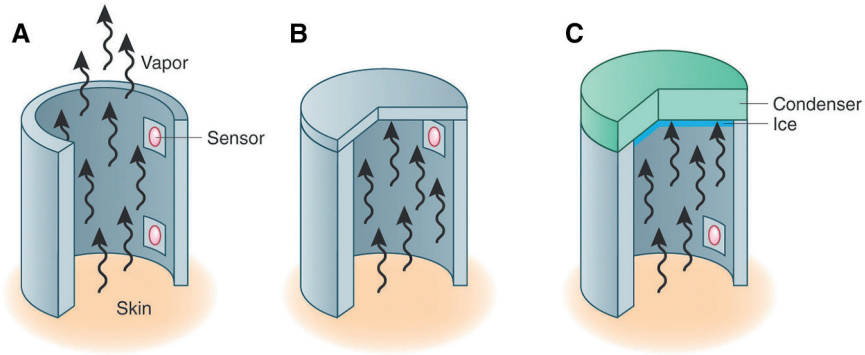

Figure 9. TEWL device principles. **A,** Open-chamber. A hollow cylinder is placed in contact with the skin, and water vapor diffuses through the open chamber. Spatially separated temperature and humidity sensors detect the humidity gradient. *Limitation:* vulnerable to environmental influences. **B,** Unventilated-chamber. The upper end of the chamber is closed, resulting in water vapor collecting in the chamber. The temperature and relative humidity sensors detect the rate of increase of relative humidity. *Limitation:* not suitable for continuous measurements. **C,** Condenser-chamber. The upper end of the chamber is closed by a condenser that removes water vapor from the chamber. Water vapor density is measured by sensors in the chamber and condenser. *Advantages:* suitable for continuous measurements, less vulnerable to environmental factors. *Reprinted with permission from Alexander et al 2018*[13]

SC hydration devices

Stratum corneum hydration is measured by analysis of electrical conductance or capacitance of the skin surface.[142] Electrical transport through keratinized tissue involves different types of electrical charge carriers of which the conduction exchange of protons along the H-bonded network of water molecules dominates, and is therefore highly dependent on water content.[144] Conductance-based instruments apply an electric current to measure the conductance of the SC in [μS]. Capacitance-based instruments apply a small oscillating electric field with low frequency to measure the dielectric constant of the skin in arbitrary units [a.u.]. See Fig. 10 for the capacitance principle of single and multi-sensor devices.[145] Current, conventional corneometers are single-sensor, only able to perform one measurement. In this thesis, also a newly developed multi-sensor device will be investigated. Corresponding to TEWL, also SC hydration shows anatomical site variation.[146,147] It is important to note that any polar substance or chemical applied to the skin surface may contribute to the electrical signal being recorded with the instrument and read as water. As water distribution of the skin surface is not completely even, due to the skin relief and variable distribution of sweat glands,[142] mapping of SC hydration is highly desirable to obtain accurate results.

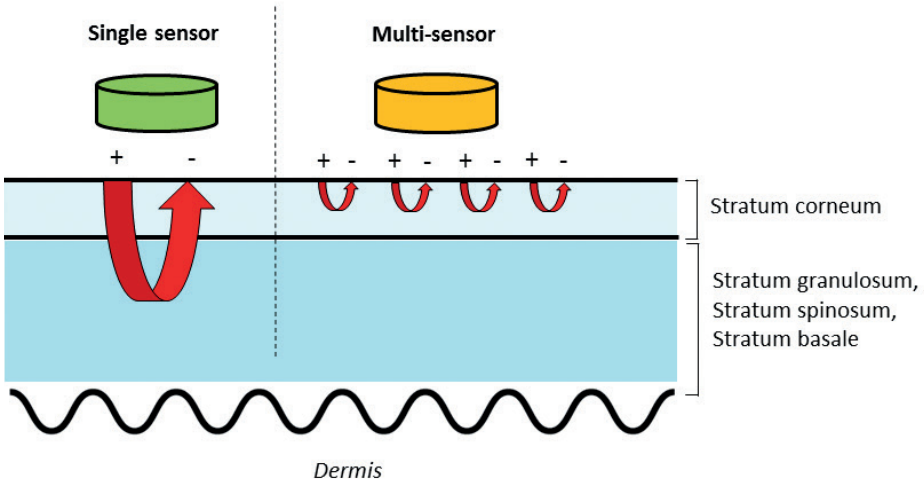

Figure 10. Capacitance-based device principle. The device produces a small oscillating electric field with low frequency and measures the dielectric constant of the skin which is placed in contact with the electrode surface. **Left**: single sensor device has one sensor, resulting in one large electrical 'loop' through the skin. *Limitation:* hydration is measured in the stratum corneum and the well-hydrated living upper dermis as well. **Right**: multi-sensor device, recently developed and not yet validated. It consists of 76 800 sensors, making multiple, small, electrical loops through the skin. *Advantage:* multiple measurements take place at once and can be averaged or can be used to get a hydration image of the stratum corneum, and hydration measurement is theoretically restricted to the 'dry' stratum corneum only.

Erythema quantification

Skin colour results from absorption and scattering of light by skin chromophores, mainly being melanin and haemoglobin.[148,149] Two principles for skin colour quantification exist; reflectance spectrophotometry and tristimulus colorimetry.[136,150] Reflectance spectrophotometry records information over a full visible spectrum of light or focuses on the quantification of reflectance at a few selected light wavelengths corresponding to the peak absorption of haemoglobin and melanin. The reflected spectrum is detected by photodiodes of the device and translated into colour values ('erythema index' or 'melanin index').[148] With tristimulus colorimetry, three wave-bands that are well separated in the visible spectrum (RGB; red, green and blue) are used, corresponding to the three types of colour sensors in the human eye. Using CIE (Commission Internationale de l'Eclarage), wavelength and intensity information are converted into three numbers, indicating how a colour of an object appears to a human observer; L^* (brightness), a^* (red-green), b^* (blue-yellow). The a^* value is frequently used as a parameter to quantify erythema in clinical studies, as it correlates almost linearly with the amount of haemoglobin in the superficial plexus.[150] Unfortunately, current colour measurement instruments only cover a small facial area, questioning representativeness of the entire face. Moreover, they require skin contact, and skin colour is easily influenced by skin pressure. These problems may be solved by computer-assisted image analysis; this will be used in this thesis.

Current limitations of biophysical devices

Besides from the aforementioned device specific limitations, current biophysical devices in general have some other drawbacks. Most devices are expensive, and their practice use is limited due to their size, weight, and speed. Generated results can be influenced by various internal and external factors such as age, anatomical site, blood flow, ambient temperature and humidity, sweating, and sun exposure.[7,13,144,149-155] Moreover, due to the differences in measurement principles it is difficult to compare results of different devices.[12] Compliance to guidelines is needed to obtain reliable and standardized measurements,[142,144,149] which requires familiarity with extensive and complicated protocols, repeated calibration, and standardized measurement conditions. As a result, biophysical analysis techniques have not had a great impact in dermatological practice so far. Biophysical tools suitable for clinical practice need to be accurate, easy-to-use, rapid, and relatively inexpensive. Ideally, this results in their routine application in dermatology clinics for objective monitoring of inflammatory dermatoses, and for improvement of personalized treatment and therapy compliance.

AIM AND OUTLINE OF THIS THESIS

Objective and noninvasive measurement methods are needed to evaluate ISDs, because current clinical assessment is subjective and biopsies are not preferred in cosmetically important regions such as the face. However, these methods are not yet embedded in daily practice due to various practical and cost-effectiveness concerns.

The main objective of this thesis was to evaluate the value of novel and conventional noninvasive imaging and biophysical tools in healthy and inflamed skin in daily clinical practice. Rosacea served as a model for inflamed skin state. This thesis primarily focused on two aims:

1. To investigate the value of imaging and biophysical tools in healthy and impaired skin barrier function.
2. To investigate the value of imaging and biophysical methods in inflamed skin, with emphasis on rosacea.

In **chapter 2** we evaluated the value of novel and combined biophysical tools to assess normal and impaired skin barrier function. In **chapter 2.1**, the Epsilon®, a new tool measuring SC hydration, was investigated to determine its use in the measurement of anatomical site variation of water content in the skin. **Chapter 2.2** focused on the feasibility of combining multiple existent biophysical/imaging devices to measure differences in skin barrier function after cream application. Furthermore, the value of the GPSkin®, a new tool to measure TEWL and SC hydration simultaneously, was evaluated to quantify an impaired skin barrier function after tapestripping (**chapter 2.3**)**.**

The purpose of **chapter 3** was to investigate the value of novel and conventional imaging and biophysical tools in rosacea for application in daily clinical practice. **Chapter 3.1** provides an extensive overview of available noninvasive objective skin measurement techniques for rosacea assessment. Based on this systematic review, we examined the value of reflectance confocal microscopy for the monitoring of rosacea during treatment with topical ivermectin (**chapter 3.2**)**.** The value of the GPSkin® was tested to monitor the skin barrier function in rosacea patients before and during treatment in **chapter 2.3.** Then, in **chapter 3.3,** we developed a computer-aided image analysis tool to facilitate quantification of facial erythema in rosacea. Next, we presented five rosacea patients with worsening of symptoms due to occlusion of the skin after use of a CPAP mask (**chapter 3.4**). In **chapter 3.5,** we evaluated the efficacy of oral β-blockers for rosacea-associated facial flushing and erythema by performing a systematic review.

Lastly, the results as described in this thesis were summarized and discussed in **chapter 4**, and future perspectives were provided.

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CHAPTER 2

Skin barrier

CHAPTER 2.1

Anatomical site variation of water content in human skin measured by the Epsilon: a pilot study

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ABSTRACT

Background

This pilot study aimed to investigate the anatomical site variation of water content of the stratum corneum (SC) on the body by measuring skin capacitance with the Epsilon, a new generation corneometer with multiple sensors. Secondly, values of the Epsilon were compared to values measured by conventional single sensor corneometers.

Methods

The hydration status of SC was measured in 15 healthy Caucasian volunteers with the Epsilon at five body sites (cheek, lower forearm, mid-calf, lower back and abdomen). Transepidermal water loss (TEWL) was measured with the Aquaflux to get more insight into the condition of the skin barrier. A literature search was performed to compare Epsilon values with conventional corneometers.

Results

The tested anatomical locations showed significant differences in water content ($P < 0.001$) with large inter-individual variations; highest values were found in the cheek (11.64ε) and lowest values in the mid-calf (4.43ε). No correlation between water content and TEWL was found. In general, Epsilon values were lower compared to values of conventional corneometers, with a similar trend.

Conclusion

This pilot study showed significant variations in water content at different skin locations measured by the Epsilon. Moreover, the Epsilon measured consistent lower values compared to single sensor corneometers. Further validation of the device is recommended.

INTRODUCTION

The stratum corneum (SC) plays an important role in skin surface management by protecting the human body against microorganisms and guaranteeing skin barrier function by regulation of moisture grade and temperature.[1-3] SC imbalance can lead to various inflammatory skin diseases, for instance constitutional eczema. The structure and barrier function of the SC can be studied noninvasively with different measurement tools; water content and transepidermal water loss (TEWL) are two commonly evaluated skin variables.[4] A corneometer measures the water content by measuring electrical capacitance of the skin surface. Until today, single sensor corneometers are used, showing significant differences in water content between various skin locations.[5-7] Recently, the Epsilon, a multi-sensor corneometer with 76800 sensors at one probe was introduced.[8] Due to this increase in sensors, multiple measurements take place at once. In addition, options for analysis are integrated in this device and water content-based images can be obtained.

To the best of our knowledge, this is the first study to investigate the anatomical site variation of water content in human skin with the Epsilon. Also, measured water content values of the Epsilon were compared to values measured by conventional single sensor corneometers by performing a literature search.

MATERIALS AND METHODS

Participants

Fifteen healthy Caucasian volunteers (nine women and six men; median age 26 years; range 21-62 years) participated in this explorative study. Informed consent was obtained. The study was approved by the local medical ethics committee and conducted according to the principles of the Declaration of Helsinki. Exclusion criteria were as follows: age < 18 years, signs of skin diseases or open wounds at the measurement sites, use of immunosuppressive medication, diagnosed with inflammatory dermatoses. Participants were asked not to use cream or body lotion at the day of measurements.

Technical device specifications

Water content of the SC was measured with the Epsilon (Epsilon E100, Biox, UK). This corneometer measures calibrated dielectric permittivity (dielectric constant, ε) through the SC and consists of a probe of 76 800 sensors with a sensing area of 12.8 x 15 mm, depth resolution of 20 µm and spacial resolution of 50 µm.[8,9] The hardware and the probe of the Epsilon are shown in Fig. 1A-D. Due to the multiple sensors, skin surface hydration can be mapped, taking skin relief and variable distribution of sweat glands into account (Fig. 1E,F). This allows measurement of more average values and

exclusion of regions with poor physical contact between sensor and skin.[10] Moreover, the Epsilon is the only corneometer with a linearized and calibrated response, allowing consistent quantitative image evaluation.[9] In this study, the standardised *Burst mode* option was used with a 5 seconds delay after first skin contact (to rule out initial variations in occlusion), a frame interval of 1-second, and a total measurement frame of 30 seconds.

To investigate the overall SC barrier function, TEWL was measured with the Aquaflux (Aquaflux AF200, Biox, UK). The closed measurement chamber of the Aquaflux contains sensors for relative humidity and temperature.[8,11] After calibration, measurements were performed with standard settings and a maximum measurement time of 80 seconds. The mean TEWL value was based on ten measure points, within a humidity degree of maximum 50%.

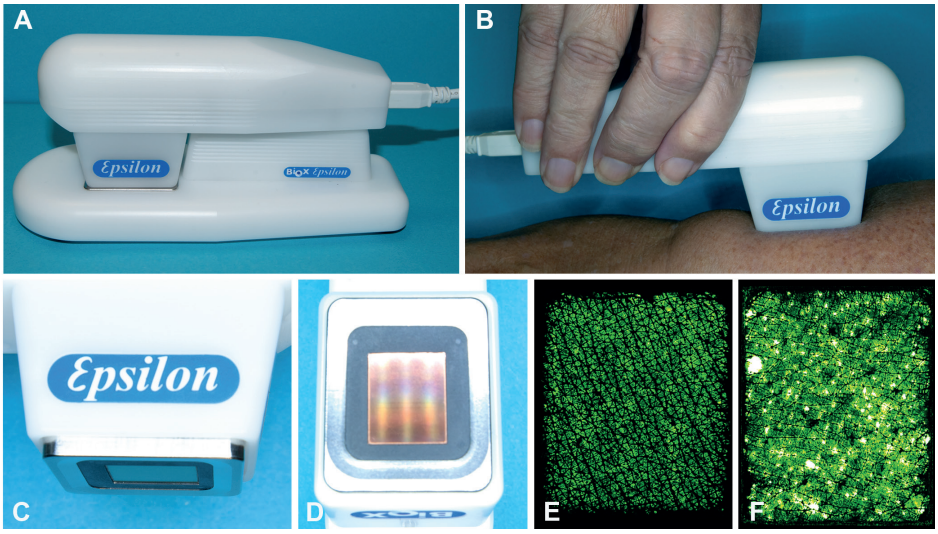

Figure 1. The Epsilon is a novel instrument for measuring near-surface dielectric permittivity (ε) and contact imaging of the skin. Its proprietary electronics and signal processing algorithms map the sensor's nonlinear signals onto a calibrated scale for measuring properties such as stratum corneum hydration. **A**, The Epsilon instrument on the parking stand. **B**, A measurement performed on the inner arm. **C**, Close-up of the Epsilon measurement head with the metal bezel. **D**, The sensor surface embedded in an epoxy frame. **E**, Typical contact image of the inner forearm skin. **F**, A contact image of the skin in the face with visible sweat gland activity.

Study procedures

In each participant, water content and TEWL were investigated at five anatomical locations; cheek, first 1/3 of the flexor surface of the lower forearm, mid-calf, lower back and abdomen (Fig. 2). For water content, one *Burst mode* measurement per body site was performed; for TEWL, the average of three measurements per body site was obtained. Standardized environmental circumstances were created; room temperature

was kept constant at 20°C and exposed skin was air-acclimatized for at least 5 minutes prior to measurements.

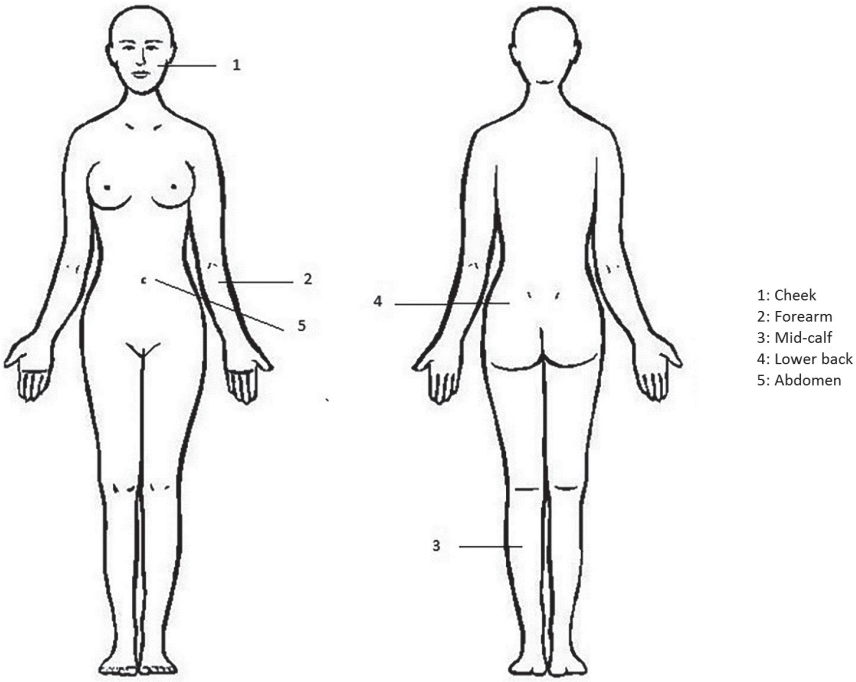

Figure 2. Measurement locations of water content and transepidermal water loss (TEWL).

Statistics

Burst mode results from the Epsilon from all body sites were used to create regression functions and *y*-axis intersections were calculated. Statistical analysis was done with SPSS Statistics 22 (IBM Corporation, Armonk, New York). A Kruskal-Wallis test with Dunn-Bonferroni post hoc method was performed to demonstrate possible differences between the water content among the body sites. A relationship between water content and TEWL was investigated using Pearson correlation analysis. Tests were performed at 0.05 significance level.

Comparison with conventional corneometers

To compare the Epsilon results with conventional corneometer values, a PubMed search was performed. Study inclusion criteria were as follows: in vivo setting, healthy/normal human skin, non-experimental setting OR use of a baseline control area in case of an intervention with topical therapies. Studied body sites preferably corresponded to the body sites chosen in this pilot study.

RESULTS

Anatomical variation in water content

The water content differed significantly between the five body sites ($P < 0.001$). As Fig. 3 shows, the cheek had the highest water content (median 11.64ε), followed by the forearm (9.35ε), abdomen (7.45ε), lower back (7.07ε) and mid-calf (4.43ε). Post hoc analysis revealed that the water content of the mid-calf was significantly lower than the water content of the cheek ($P < 0.001$) and the forearm ($P < 0.001$). Additionally, a large interindividual variation in water content among the various skin locations was seen. There was no significant correlation between water content (measured by Epsilon) and TEWL ($r = 0.194$, n = 75, $P = 0.095$).

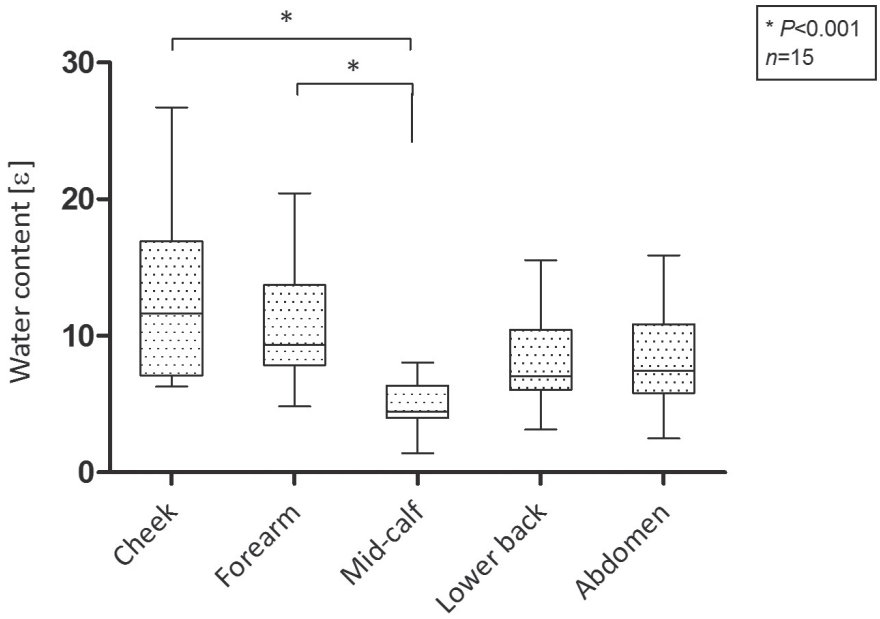

Figure 3. Water content at five body sites measured with the Epsilon. The values are expressed as median with interquartile range and range (minimum-maximum).

Comparison with conventional corneometers

Table 1 shows literature-based reference values of the water content with single sensor corneometers. In general, single sensor corneometers showed higher water content values than the Epsilon. In line with the Epsilon, also conventional corneometers measured lower values of the calf compared to the cheek and forearm.

Table 1. Overview of water content values measured with single sensor corneometers.

Study	Device	Population	Skin location			
			Forehead (a.u.)	Cheek (a.u.)	Forearm (a.u.)	Calf (a.u.)
O'goshi et al[5]	Corneometer CM820 Corneometer CM820 Corneometer CM810	53 healthy volunteers	74 (38-122)[a] 72 (29-113)[a] 78 (41-131)[a]	75 (37-100) 74 (24-96) 81 (33-104)	65 (43-115) 65 (50-100) 71 (52-113)	50 (28-90) 49 (29-90) 50 (27-95)
Egawa et al[12]	Corneometer CM825	45 healthy volunteers		*Winter:* 36 ± 11[b] *Spring:* 37 ± 11[b] *Autumn:* 48 ± 9[b] *Summer:* 50 ± 11[b]	*Winter:* 37 ± 9 *Spring:* 37 ± 6 *Autumn:* 38 ± 10 *Summer:* 47 ± 8	
O'goshi et al[6]	Skicon-100[c] Skicon-200EX	26 healthy volunteers		73 ± 52 (16-369)[a] 151 ± 86 (27-414)[a]	50 ± 27 (11-123) 80 ± 56 (27-272)	26.5 ± 31 (3-153) 37.5 ± 71 (9-425)
Algiert-Zielińska et al[13]	Corneometer CM825	10 healthy volunteers	*Left side:* 46.67 ± 10 (28.1-65.35)[b] *Right side:* 51.04 ± 12.50 (30-64.64)[b]	*Left side:* 39.77 ± 13.78 (19.60-59.52) *Right side:* 44.2 ± 12.65 (27.18-66.72)		
Kleesz et al[14]	Corneometer CM825	125 healthy volunteers	75 ± 13[b]	72 ± 16	62 ± 13	58 ± 10

Table 1. Continued.

Study	Device	Population	Skin location			
			Forehead (a.u.)	Cheek (a.u.)	Forearm (a.u.)	Calf (a.u.)
de Farias Pires et al[15]	Corneometer CM820	1339 healthy volunteers		*Female:* 37 (9-78)[a] *Male:* 28 (5-66)[a]	*Female:* 32 (10-56) *Male:* 27 (2-56)	
Young et al[16]	Corneometer CM825	21 healthy volunteers			25 (24.5-25.5)[¥]	
Marrakchi et al[17]	Corneometer CM 420	20 healthy volunteers	*24-34 years:* 89.33 ± 12.7[b] *66-83 years:* 76.90 ± 18.2[b]	*24-34 years:* 87.40 ± 12.4 *66-83 years:* 84.00 ± 16.9	*24-34 years:* 81.70 ± 11.1 *66-83 years:* 95.10 ± 7.1	
Fluhr et al[18]	Corneometer CM825	7 healthy volunteers			46.4 ± 6.5[b]	
Lodén et al[19]	Corneometer CM820	17 healthy volunteers			70 (69-82)[a]	
Esposito et al[20]	Corneometer CM820	10 healthy volunteers			22 (20-24)[b]	
Cheng et al[21]	Corneometer CM825	30 healthy volunteers	55 ± 9[b]	55 ± 8		

Table 1. Continued.

Study	Device	Population	Skin location			
			Forehead (a.u.)	Cheek (a.u.)	Forearm (a.u.)	Calf (a.u.)
Hillebrand et al[22]	Corneometer 820PC	602 healthy females		*5-15 years:* 59.90 ± 11.7[b] *25-35 years:* 76.87 ± 10.0[b] *45-54 years:* 78.74 ± 10.8[b] *55-65 years:* 77.48 ± 11.9[b]	*5-15 years:* 48.43 ± 4.30 *25-35 years:* 54.82 ± 6.0 *45-54 years:* 57.59 ± 6.7 *55-65 years:* 60.03 ± 6.2	
Agache et al[23]	Corneometer CM820	20 healthy volunteers			55.45 ± 2[b]	
Richters et al[24]	Corneometer CM825	30 volunteers with non-sensitive skin		37.9 (9.0-62.3)[a]	31.7 (19.4-55.6)	

[a] Median ± SD (range). [b] Mean ± SD (range). [c] Measures skin conductance; close correlation to skin capacitance.[25]
a.u., arbitrary units. Words in italic describe specific conditions/subgroups studied.

DISCUSSION

This pilot study is the first to measure water content of the SC in different body sites with the Epsilon. Our findings showed significant differences among the body sites, in correspondence with previous studies that also showed this trend.[5-7,14,26] Many factors could influence these regional differences, for example, variations in presence of sebaceous glands and lipids, natural moisturizing factor (NMF), size of corneocytes, exogenous compounds on skin surface and occlusion.[4,14,27] Also SC thickness variation could play a role; the smallest SC cell number is found in genital skin, followed by the face, neck, scalp, trunk, extremities and palmoplantar region.[4,27,28] Moreover, skin surface hydration gradually increases in deeper layers of the SC, reaching a certain high level in the fully hydrated epidermis.[12,25] It is therefore more likely to measure water content in deeper and more hydrated layers of skin with thinner SC (e.g., cheek), resulting in higher values.

Another important finding was that water content values of the Epsilon were lower compared to values of conventional corneometers. First, it is important to bear in mind that Epsilon measurement units are displayed using a calibrated dielectric permittivity scale (ε) rather than an arbitrary scale (a.u.) as used in conventional corneometers. As both instrument types use the same capacitance measurement principle, they should correlate well; this was already shown by one-to-one testing of both devices on the volar forearm of healthy volunteers.[29] With the multisensory character of the Epsilon, the sensing depth will probably be more superficial compared to conventional corneometers, which make one big electrical loop through the skin. This increases the chance that Epsilon measurements are confined to the relatively "dry" SC only. Another advantage of the Epsilon is the *Burst mode* setting, correcting for time-dependent skin occlusion differences, while conventional corneometers perform single time point measurements. Thirdly, due to the "skin mapping" character of the Epsilon, the number of values in one measurement can be averaged. All of the above could potentially lead to more accurate water content values.

The large interindividual variation of water content among the different skin locations could be influenced by individual parameters, for example, age, gender and lifestyle.[4,5,26,30] This was not studied in more detail because of the explorative character of this pilot.

Interestingly, no correlation was found between water content and TEWL. One would expect that TEWL increases in a disrupted skin barrier, resulting in lower water content, and vice versa. However, also previous studies showed no or only weak correlations between these two measurements.[24,31] As mentioned earlier, other factors besides from TEWL and water content seem to be responsible for alterations of skin barrier function.

Despite the relatively small number of volunteers, these pilot results are promising. Larger populations of healthy volunteers and patients should be investigated for further validation of the Epsilon. This could elucidate the potential of this device for diagnosis and/or therapeutic monitoring of subjects having skin diseases with decreased barrier function, for example, inflammatory dermatoses. It would also be interesting to study possible interactions between water content and other noninvasive skin barrier measurements (e.g., NMF and sebum levels[24,25]) and the possible impact of inter-seasonal fluctuation on skin condition.

In conclusion, we found significant regional differences in water content in human skin measured by the Epsilon. Moreover, the Epsilon measures lower water content values compared to conventional single sensor corneometers and these values show an equal trend in differences of water content among different body sites. It is recommended to investigate these findings in a larger population for further validation of the Epsilon and to determine if this device can be implemented into the clinical setting.

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CHAPTER 2.2

Noninvasive skin barrier assessment: multiparametric approach and pilot study

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Cosmetics 2019;6:20

ABSTRACT

The epidermal barrier function is disrupted in various inflammatory skin diseases. Accurate methods to measure skin barrier function are needed to assess the effect of therapeutic agents. Therefore, we developed a noninvasive multiparametric approach to measure four different parameters regarding the skin barrier. In the current pilot study, we evaluate this method in 14 healthy volunteers. We assessed erythema, transepidermal water loss (TEWL), water content, and epidermal thickness at both cheeks before and 30 minutes after application of Lanette and Vaseline-Lanette cream. For this, we used spectrophotometry, the Aquaflux device, the Epsilon device, and reflection confocal microscopy, respectively. Stratum corneum (SC) thickness was significantly increased after application of both creams ($P < 0.05$), and this increase was larger after Lanette cream compared to after Vaseline-Lanette cream ($P = 0.035$). Erythema, TEWL and water content did not significantly change after cream application. Our multiparametric approach is promising and offers a feasible and practical way to quickly obtain multifaceted information about skin barrier function. Further exploration of this approach after prolonged use of cream and in conditions of disrupted skin barrier are recommended areas for future research.

INTRODUCTION

The skin barrier acts as a first-order shield to protect the human body against micro-organisms, ultraviolet (UV) light, and mechanical injury, and also helps regulate temperature and water flux.[1,2] It is predominantly formed by the outermost layer of human epithelial cells, the stratum corneum (SC). [3] The skin barrier function is disrupted in inflammatory skin diseases like rosacea and atopic dermatitis.[4-10] An impaired skin barrier is characterized by increased transepidermal water loss (TEWL) and reduced epidermal hydration, and predisposes one to cutaneous inflammation.[2] Conversely, skin moisturizers can improve the skin barrier and help prevent inflammation.[2,9,11-14] However, not all moisturizers are beneficial to the skin barrier, and, for unknown reasons, some topical ointments may exacerbate symptoms in inflammatory skin disease.[15,16]

The skin barrier function is most often assessed using methods that quantify water content and TEWL. Unfortunately, these are single-device methods that measure only one or a few parameters, while a multiparametric approach is preferred to better assess subtle features of skin damage and restoration in the very complex environment of the skin. In order to better understand the mechanisms of skin therapeutic agents, accurate, objective, and reliable methods to measure skin barrier function are needed. Besides water content and TEWL, other skin parameters may influence skin barrier function, such as natural moisturizing factor (NMF), SC thickness, dermal vasodilatation (erythema), intracellular lipids, and pH.[2,4,15] A wide array of noninvasive biophysical and imaging methods is available to assess most of these parameters.[17] In the current pilot study, we demonstrate the feasibility of combining a quartet of biophysical/imaging devices to measure the following four skin parameters noninvasively; erythema, TEWL, water content, and epidermal thickness. These four skin parameters were selected for their ability to be measured in the face and their practical use. To the best of our knowledge, this combination of parameters has never been studied before in skin barrier assessment. We quantify these parameters before and after application of two different creams.

MATERIALS AND METHODS

Study subjects

Fourteen healthy Caucasian volunteers (12 women and two men; mean age, 24 years; range, 21-26 years) with skin types I to III were included in the study after providing written informed consent. The study was approved by the local medical ethics committee and conducted according to the principles of the Declaration of Helsinki. Measurements were performed in October 2018 at the department of Dermatology, Radboud University Medical Center, Nijmegen, the Netherlands. The

following exclusion criteria were adopted: age < 18 years, signs of skin disease at measurement sites, known hypersensitivity reaction to Vaseline-Lanette cream, use of immunosuppressive medication, or a diagnosis of inflammatory skin disease. Subjects did not use cream, body lotion, make-up, or perfume at the day of measurements and refrained from physical exercise within three hours before measurements.

Products

We applied two widely used vehicles that are able to penetrate the skin within 30 minutes: Lanette cream and Vaseline-Lanette cream (Table 1).

Table 1. Ingredients of investigational products.

Product	Ingredients (in order of percentage of cream content)
Lanette cream I FNA	Purified water, decyloleate, cetostearyl alcohol, B emulsifying, sorbitol solution, sorbic acid
Vaseline-Lanette cream FNA	Cetostearyl alcohol, B emulsifying, cetiol V, sorbic acid, sorbitol solution, white petrolatum (vaseline), purified water

FNA, Formularium der Nederlandse Apothekers

Study procedures and technical device specifications

Measurements of the four skin barrier parameters (erythema, TEWL, water content, and SC and epidermal thickness) were performed on both cheeks. First, measurement locations were demarcated with a surgical pen (Fig. 1). Table 2 shows a detailed overview of the measurement principles of the four devices used to measure the skin barrier parameters. Facial skin was acclimatized to the ambient air for at least 10 minutes before the start of the measurements, and room temperature and air humidity were kept constant at 20-23°C and 38-55%, respectively. Volunteers were laid down in supine position during acclimatization period and measurements to prevent possible orthostatic interactions.

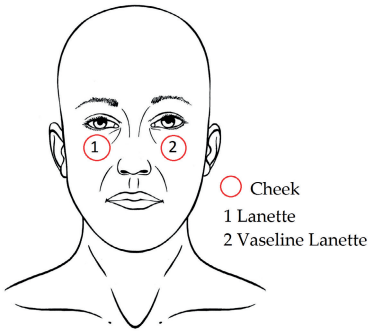

Figure 1. Measurement locations for erythema, transepidermal water loss, water content and skin thickness per cheek.

Table 2. Overview of the four devices used for the skin barrier function measurements.

Device ***Skin parameter assessed***	**Measurement principle**	**Output**	**Measurement time**	**References**
Spectrophotometer (Konica CM-2600d) *Erythema*	Intense white light from a xenon lamp is emitted by a probe. The device is placed onto the skin. The color of the reflected light is analyzed by three photocells filtering the primary colors (blue: 450 nm, green: 550 nm, red: 610 nm). Allows measurement of the absorbance and reflectance spectrum in the 400-700 nm range.	Color expressed as L*a*b color space. This is a three-dimensional coordinate system with an L*axis (brightness) and two orthogonal axes representing chromaticity, namely a*axis (red-green) and b*axis (yellow-blue). The a* value from the measurement locations is obtained after repeated calibration on a white surface before each measuring session (0.00).	1.5 seconds	17-19
Aquaflux (Biox) *TEWL*	A probe consisting of a closed chamber with a condenser and sensors for relative temperature and humidity is applied onto the skin surface. The flux of vapor is calculated due to increasing temperature and humidity rate.	Flux density of water vapor ($g/m^2/h$).	Max. 180 seconds	20-22
Epsilon (Biox) *Water content*	A probe consisting of 76 800 sensors with a sensing area of 1.3 x 1.5 cm, a resolution of 50 µm, and a measurement depth of 20 µm is placed onto the skin. The electrical capacitance of the skin surface is calculated.	Calibrated dielectric permittivity (ε) through the SC. Moreover, capacitive contact images can be obtained (brighter color = higher dielectric constant; darker color = lower dielectric constant) for skin surface hydration mapping, taking skin relief and variable distribution of sweat glands into account.	30 seconds	23-25

Table 2. Continued.

Device ***Skin parameter assessed***	**Measurement principle**	**Output**	**Measurement time**	**References**
Reflectance confocal microscope (VivaScope 1500) Thickness of SC and *viable epidermis*	Laser light at 830 nm is focused onto the skin with maximum imaging depth of 200 µm below skin surface (papillary dermis). Due to different refractive indexes between the cell structures and the surrounding tissue, en face images at 30x magnification of morphological and cellular resolution are obtained. Horizontal resolution: 0.5-1 µm, vertical resolution: 3-5 µm.	Black and white images showing skin morphology. Options: - VivaCam: dermoscopic image. - Confocal: basic image of 500 µm x 500 µm. - VivaBlock: multiple confocals acquired at the same level, stitched together to create one larger image (max. 8 mm x 8 mm). - VivaStack: multiple confocals along depth at a certain location, with interval steps of 3-5 µm - Movie: eg, to view blood flow in the superficial dermis.	2-3 minutes	[17,26,27]

SC, stratum corneum. TEWL, transepidermal water loss

Firstly, erythema was measured with a spectrophotometer (CM-2600d, Konica Minolta, Ramsey, NJ, USA). Using the principle of diffuse reflectance spectroscopy, light absorption from primary chromophores in the skin (melanin, oxyhemoglobin, and deoxy-hemoglobin) was measured.[19] Before each measuring session, calibration to a standard white plate provided with the meter was performed. After pressing the calibrate button, three calibration measurements were automatically taken. Next, three measurements on each cheek were taken; the device was lifted and gently reapplied between each recording. The average of the three consecutive erythema measurements was automatically calculated by the device. The measuring head was kept steady and perpendicular to the skin surface with very light pressure against the skin during measurements to prevent minor venous congestion. More detailed information about measurement requirements can be found in Fullerton et al.[18]

Next, TEWL was measured with the Aquaflux (AF200, Biox, London, UK). After calibration, three measurements per cheek were performed with standard settings and a maximum measurement time of 180 seconds. The average of the three measurements per cheek side was calculated. Also, the Aquaflux device was kept steady and perpendicular to the skin surface with very light skin pressure during measurements.

Thirdly, water content was determined by performing one measurement per cheek with the Epsilon (E100, Biox, London, UK), a new-generation corneometer. Compared to conventional single sensor corneometers, the Epsilon contains 76 800 sensors; thus, multiple measurements take place at once.[25] Moreover, analysis software is integrated in the device, and water-content-based images can be obtained. The *Burst mode* option was used with a 5-seconds measurement delay after first skin contact to rule out initial variation in skin occlusion. A frame interval of 1 second and a total measurement window of 30 seconds was selected. To keep contact with the skin surface, moderate pressure was applied.

Lastly, the reflectance confocal microscope (RCM; Vivascope 1500, Mavig, Munich, Germany) was used to determine skin thickness. For each cheek, one dermoscopic image (VivaCam) was obtained and used as map. Next, five vertical mappings (VivaStacks) were performed at the center and at the four outer corners of the image (Fig. 2). Series of images of 0.5 x 0.5 mm were obtained, starting from the skin surface up to a depth of 100 µm with interval steps of 3 µm. With a standardized protocol (Fig. S1, Supplementary Materials) all VivaStack images were evaluated by one researcher to determine thickness of the SC and the viable epidermis (without SC).

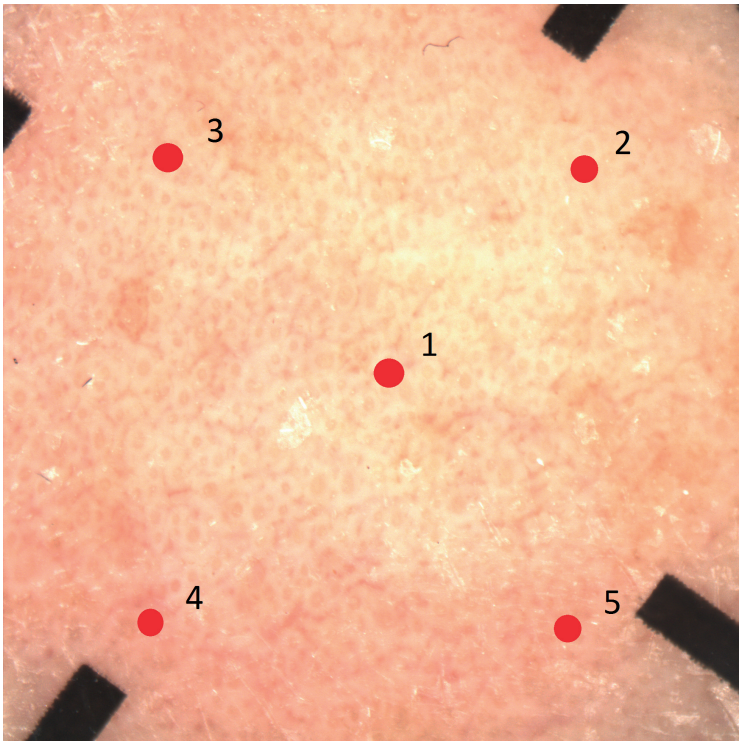

Figure 2. Dermoscopic image of the cheek made by using the reflectance confocal microscope. The red dots show the exact locations were the VivaStacks were performed.

After baseline measurements, the Lanette cream was applied to the right cheek, and the Vaseline-Lanette cream to the left cheek (Fig. 1). One finger-tip unit (FTU) of cream was used per cheek side, according to the finger-tip method.[28] One FTU corresponds to 0.5 g. After 30 minutes, the creams were completely absorbed into the skin, and all skin parameters (erythema, TEWL, water content, epidermal thickness) were repeated as described above.

Analysis

Burst mode values from the Epsilon measurements were used to create regression functions, and intersections with the *y*-axis were calculated. Statistical analysis was performed with SPSS (SPSS statistics 25, IBM Corporation, USA). Possible baseline differences of the skin barrier parameters between the right and left cheek were explored with the Wilcoxon signed-rank test. The Wilcoxon signed-rank test was also used to demonstrate significant differences between skin barrier parameters before and after application of both creams. A relationship between the skin parameters and environmental factors was investigated using Spearman's correlation. *P*-values below 0.05 were considered significant.

RESULTS

Compared to baseline, the thickness of the SC increased significantly after application of both creams ($P < 0.05$, Table 3 and Fig. 3). The absolute difference of the SC thickness before and after application was significantly larger for Lanette cream compared to Vaseline-Lanette cream ($P = 0.035$). Fig. 4 shows no morphological changes of corneocytes in the SC after cream application with RCM. Erythema, TEWL, water content and thickness of the viable epidermis did not significantly change after application of Lanette cream and Vaseline-Lanette cream. Additionally, relatively large interindividual variations among skin parameters were seen. Values of all skin parameters at baseline compared to 30 minutes after cream application per subject are displayed in Fig. S2 and Table S1 (Supplementary Materials). No significant differences in median baseline values of erythema, TEWL, water content and thickness of the SC and the viable epidermis were found between the right and left cheek among subjects ($P > 0.05$ for all values).

There were no significant relationships between the four skin parameters. Additionally, no biologically relevant correlations between the skin parameters and environmental factors were found (data now shown).

Table 3. Skin parameters measured at baseline and at 30 min after cream application.

Skin parameter	**Lanette**			**Vaseline-Lanette**		
	Baseline	30 minutes after cream application	*P*-value	Baseline	30 minutes after cream application	*P*-value
Erythema, *a**	48.40 (46.20-50.80)[a]	48.00 (46.10-50.60)	.362	47.95 (44.60-49.80)	48.05 (43.20-49.80)	.965
TEWL, *g/m²/h*	19.28 (14.05-25.31)	19.09 (15.87-26.71)	.363	18.81 (13.67-27.22)	19.00 (14.61-24.54)	.300
Water content, *ε*	20.16 (14.86-33.94)	23.29 (15.89-28.33)	.730	21.11 (16.31-47.02)	21.79 (14.03-40.02)	.363
Thickness SC, *μm*	5.25 (3.71-7.42)	9.90 (5.15-13.92)	**.001[b]**	4.64 (3.09-11.34)	6.49 (4.12-9.90)	**.016[b]**
Thickness viable epidermis, *μm*	54.59 (48.86–64.32)	52.96 (45.61–60.61)	.551	54.22 (45.64–59.31)	54.12 (42.26–57.91)	.638

[a] Median (range). [b] $P < 0.05$ (baseline vs. 30 minutes after cream application).

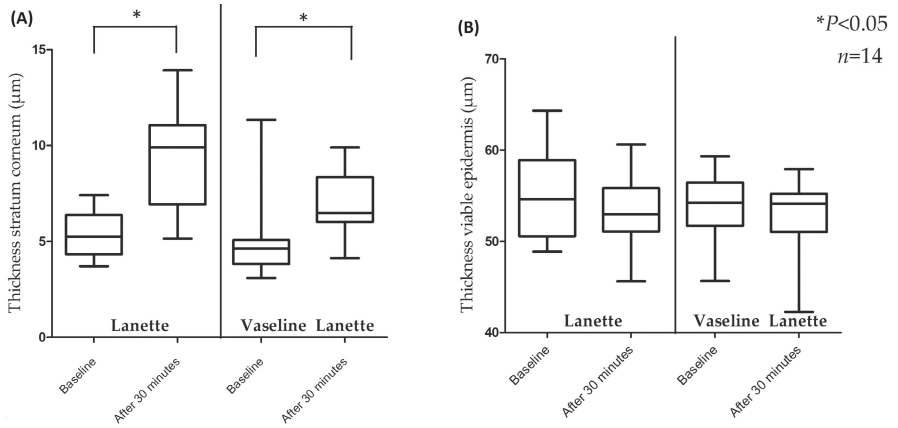

Figure 3. Thickness of stratum corneum (**A**) and viable epidermis (**B**) measured by the reflectance confocal microscope at baseline and 30 minutes after cream application. Values are expressed as medians with interquartile ranges and ranges (minimum–maximum).

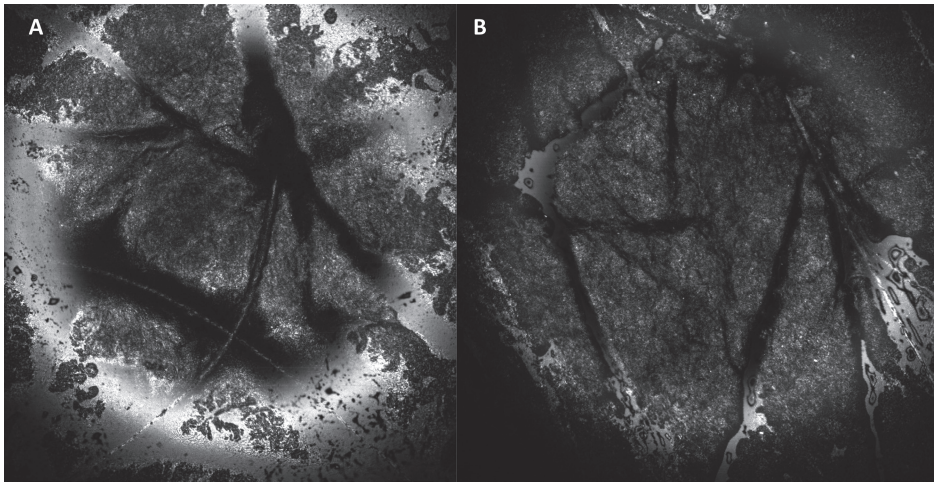

Figure 4. Representative reflectance confocal microscopy images of the stratum corneum at baseline (**A**) and 30 minutes after cream application (**B**).

DISCUSSION

This is the first study that combines the noninvasive measurements of erythema, TEWL, water content, and epidermal thickness to assess the effects of indifferent creams on the healthy skin barrier. Cream application did not affect erythema, TEWL and water content 30 minutes after application on the cheeks, but did lead to increased SC thickness. The four modalities used in this study (spectrophotometer, Aquaflux, Epsilon, RCM) can monitor the same facial skin location over time without discomfort, damage or alteration. All devices are portable and easy to use, and measurements are painless and rapid (15 minutes total).

To measure TEWL, we used the Aquaflux, a condenser closed-chamber system. Earlier research showed that this chamber system is the most sensitive TEWL system, allowing continuous measurements, and is the least vulnerable to environmental influences.[21,29] However, some drawbacks have to be taken into account. The device glides easily onto skin during measurements; manual fixation is recommended, which we did. Start-up time including calibration takes relatively long (15 minutes); thus, this should be prepared before measurements. Additionally, repeated measurements on exactly the same location are challenging due to the small probe. This might have resulted in small intraindividual variations, because TEWL values can vary between facial areas.[30-32]

Water content was measured with the Epsilon, a state-of-the-art corneometer using multi-sensor skin mapping technology with correction for skin occlusive effects.[23,25] The sensing depth of the Epsilon is restricted to the less hydrated SC, in contrast to earlier model conventional corneometers that measure the deeper, more hydrated epidermis;[32,33] this potentially leads to more accurate water content values. The Epsilon showed significant variations in water content at different anatomical skin locations;[25] however, the size of the probe head (approx. 4 x 3 cm) restricts measurements to non-recessed body parts.

Lastly, epidermal thickness was assessed using RCM. The images with cellular resolution allow very detailed measurements of the epidermal layer.[27] Earlier work showed very good correspondence of RCM and histology for epidermal thickness measurements.[34] Limitations for use in daily practice are the high device cost and limited imaging depth, as imaging resolution decreases substantially below 150 µm (superficial dermis). In addition, measuring facial skin may be challenging. The transition from epidermis to dermis differs from other anatomical locations; in facial skin, interpapillary processes do not or barely exist due to sun exposure, and the dermal-epidermal junction is low refractive in skin types I/II because of limited melanin content in the basal layer.[35,36] So, knowledge about skin morphology is recommended for evaluation of the images. Of all imaging tools, RCM is superior in noninvasive skin thickness measurements. Possible alternatives are high-frequency ultrasound, near-infrared spectroscopy,

optical coherence tomography, or Raman spectroscopy; however, these devices have lower resolution and are also expensive.[17,37-39] Moreover, they may not all be suitable for facial imaging due to their size (e.g., Raman spectroscopy)

It is already known that application of moisturizers increases SC thickness, probably due to absorption of water (indicating increased water-binding capacity), corneocyte swelling or reorganization in the lipid bilayer.[15,40,41] Surprisingly, we did not measure increased water content after cream application. Moreover, the RCM images showed no morphological changes of corneocytes in the SC after cream application. This suggests that increased SC thickness in our study may not be due to increased hydration or corneocyte swelling, but rather due to cream components other than water.[23] Alternatively, it could be that we did not find increased SC water content because water may have diffused into deeper layers of the epidermis in the 30 minutes after cream application, or it could be that repeated use of moisturizers is needed to significantly increase epidermal water content.[14,15,40,42-44]

Given the explorative design, our study consisted of a small number of young volunteers and female predominance. It would be interesting to investigate our method with prolonged use of topical creams in a larger population, at various facial locations and to include an untreated reference area. Moreover, measurements in subjects after controlled skin barrier perturbation (e.g., tapestripping[21]) or in patients with disturbed skin barrier function could provide more insight into the effect of cream application. It would also be interesting to expand our method with the other skin-barrier-related parameters (sebum, pH).

In conclusion, we combined the noninvasive measurements of erythema, TEWL, water content and epidermal thickness to assess the effect of different creams on the healthy facial skin barrier. Cream application did not affect erythema, TEWL and water content, but did lead to increased SC thickness. Our multiparametric approach is promising and offers a feasible and practical way to quickly obtain multifaceted information about skin barrier function. Further exploration of this approach after prolonged use of cream and in conditions of disrupted skin barriers are recommended areas for future research.

SUPPLEMENTARY MATERIALS

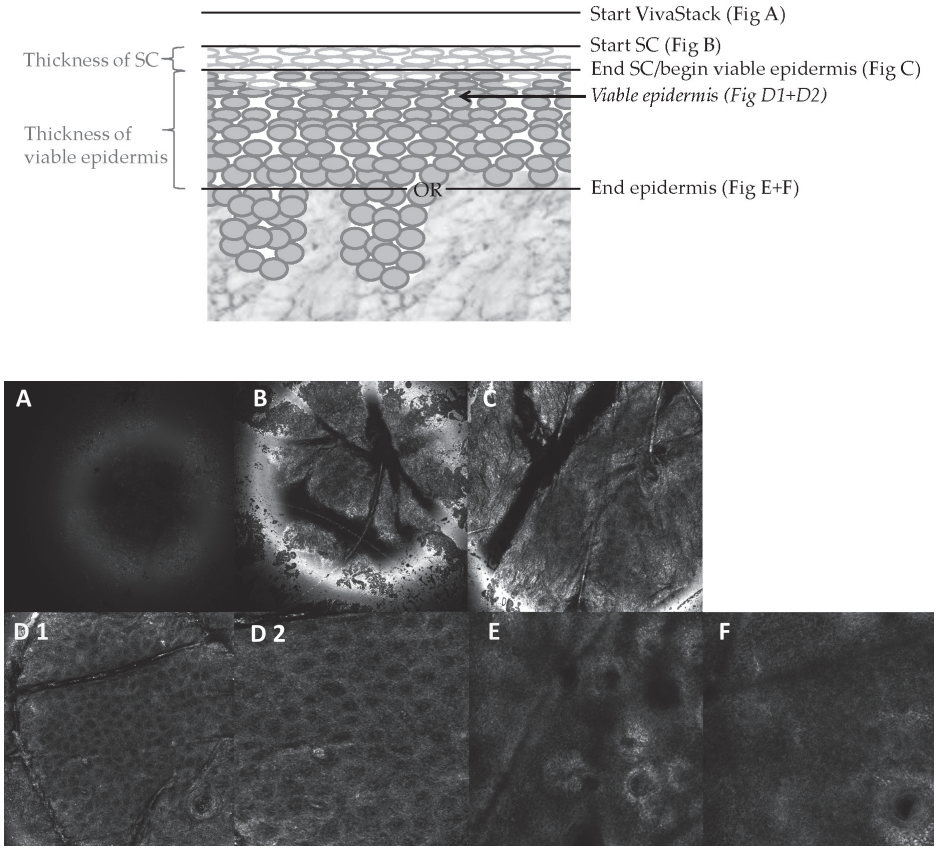

Figure S1. Protocol used for VivaStack evaluation to determine thickness of the stratum corneum (SC) and epidermis with reflectance confocal microscopy (adapted from Ardigo et al[26]), with examples to distinguish the boundaries between the different skin layers. The central part of the images was used to determine the transition of the different skin layers, to rule out variation in depth (skin layers) within the images. **A,** Image acquisition should start above the beginning of the SC; the plastic window is seen as a bright round circle. **B,** Beginning of SC, the first image with bright, highly reflective anuclear cells is visible. **C,** The last image with completely bright SC is considered as the end of the SC/begin of viable epidermis. **D1,** Part of the viable epidermis with nucleated cells (honeycombed pattern) at centre of the image. **D2,** Close-up of honeycombed pattern as described in D1. **E,** First appearance of the papillary dermis at the centre of the image is considered as the end of the epidermis. **F,** Disappearance of the honeycombed pattern at the centre of the image is also considered as the end of the epidermis.

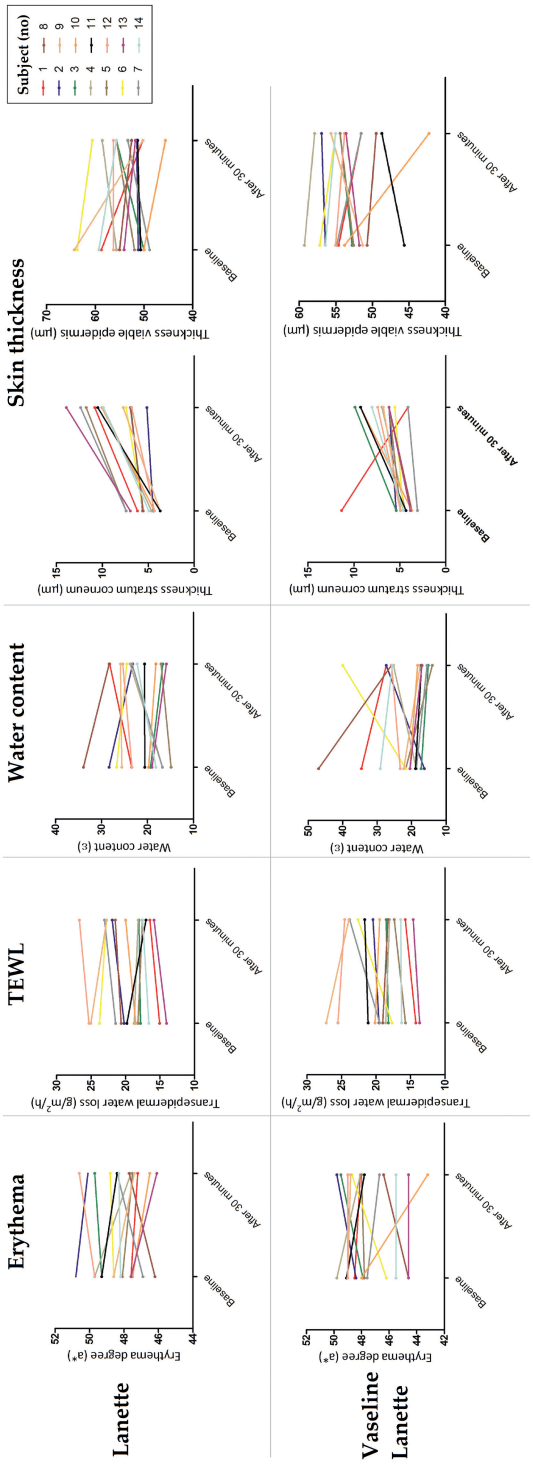

Figure S2. Differences in erythema, transepidermal water loss, water content, and skin thickness between baseline and 30 min after cream application per volunteer.

Table S1. Differences in erythema, transepidermal water loss, water content, and skin thickness between baseline and 30 min after cream application per volunteer.

	Subject (no)	Skin parameter				
		Erythema (a*)	TEWL ($g/m^2/h$)	Water content (ε)	Thickness SC (µm)	Thickness viable epidermis (µm)
Lanette	1	47.60[a] / 47.20[b]	15.05 / 16.47	23.43 / 28.21	6.18 / 10.83	58.76 / 50.25
	2	50.80 / 50.10	20.20 / 21.94	28.36 / 23.22	4.47 / 5.15	51.19 / 51.55
	3	49.30 / 49.70	17.84 / 18.06	19.34 / 16.63	5.63 / 6.90	50.03 / 55.65
	4	49.70 / 47.60	18.75 / 18.18	16.73 / 23.80	4.64 / 9.89	55.66 / 58.60
	5	48.10 / 47.50	18.21 / 17.56	14.86 / 17.03	7.42 / 11.75	51.96 / 55.66
	6	48.60 / 48.80	23.79 / 22.77	26.71 / 24.51	5.56 / 7.42	63.71 / 60.61
	7	46.90 / 48.40	21.44 / 21.44	16.73 / 23.35	7.42 / 12.37	48.86 / 53.34
	8	46.20 / 47.70	20.65 / 21.49	33.94 / 28.33	5.56 / 6.96	55.05 / 52.57
	9	48.60 / 47.40	25.04 / 22.74	25.58 / 25.89	3.71 / 7.73	64.32 / 50.25
	10	47.50 / 46.50	18.53 / 19.99	19.73 / 18.17	4.33 / 10.05	50.10 / 45.61
	11	49.30 / 48.40	19.81 / 17.04	20.59 / 20.65	3.71 / 10.51	50.72 / 51.34
	12	49.70 / 50.60	25.31 / 26.71	23.37 / 25.38	4.33 / 6.80	56.28 / 56.29
	13	47.60 / 46.10	14.05 / 15.87	19.02 / 15.89	6.96 / 13.92	54.12 / 51.80
	14	48.20 / 48.30	16.62 / 17.53	18.17 / 22.24	4.94 / 9.90	59.29 / 55.66
Vaseline-Lanette	1	48.50 / 48.10	14.23 / 15.76	34.60 / 27.38	11.34 / 4.12	54.63 / 51.54
	2	48.40 / 49.80	19.56 / 20.44	16.31 / 27.38	5.41 / 6.18	56.44 / 56.95
	3	47.90 / 49.50	18.20 / 18.52	17.25 / 15.17	5.41/ 9.90	52.78 / 54.42
	4	49.80 / 48.10	18.59 / 18.04	18.67 / 25.30	4.39 / 6.96	59.31 / 57.91
	5	47.80 / 48.00	15.73 / 17.35	21.72 / 14.03	4.94 / 6.18	52.58 / 54.43
	6	46.20 / 48.70	17.68 / 22.61	21.72 / 40.02	4.64 / 5.56	57.21 / 55.05
	7	47.60 / 46.70	19.48 / 23.83	18.65 / 15.56	3.09 / 4.12	54.89 / 51.54
	8	44.60 / 46.40	19.03 / 18.25	47.02 / 25.87	3.71 / 6.18	50.72 / 49.49
	9	46.40 / 48.80	27.22 / 23.94	22.27 / 17.55	4.95 / 6.80	51.33 / 55.67
	10	48.00 / 43.20	20.14 / 19.47	20.51 / 18.28	3.71 / 9.28	53.81 / 42.26
	11	49.10 / 47.80	21.15 / 21.64	18.87 / 17.16	4.33 / 9.28	45.64 / 48.70
	12	49.00 / 49.00	25.53 / 24.54	23.32 / 25.83	4.94 / 7.42	55.05 / 53.81
	13	44.60 / 44.60	13.67 / 14.61	20.47 / 17.03	3.86 / 6.18	51.80 / 53.61
	14	45.50 / 45.50	16.29 / 16.43	29.10 / 25.61	4.64 / 8.04	56.44 / 55.05

[a] Baseline. [b] 30 min after cream application.

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CHAPTER 2.3

Value of GPSkin for the measurement of skin barrier impairment and for monitoring of rosacea treatment in daily practice

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ABSTRACT

Background

Stratum corneum hydration (SCH) and transepidermal water loss (TEWL) provide useful information about skin barrier function. This study aimed to determine the value of GPSkin Pro, a new handheld device determining both SCH and TEWL, to measure skin barrier impairment and to monitor barrier function in rosacea in daily practice.

Materials and Methods

Two pilots were performed. *Pilot 1*: in 27 healthy participants, GPSkin SCH and TEWL were compared to Aquaflux and Epsilon values at the forearm before and after skin barrier perturbation via tapestripping. Moreover, GPSkin values were measured at both cheeks without intervention. *Pilot 2*: in 16 rosacea patients, GPSkin measurements were performed at the forearm, and at both cheeks before and during anti-inflammatory treatment. They were compared to clinical symptoms and to GPSkin values from pilot 1.

Results

Pilot 1: after merging data from before and after tapestripping, a strong correlation was observed between GPSkin TEWL and Aquaflux (R_s = 0.9256), and GPSkin SCH and Epsilon (R_s = 0.8798). *Pilot 2:* SCH was significantly lower at the cheeks of rosacea patients compared to controls, with a normalizing trend during successful treatment. TEWL was comparable among patients and controls and did not change during treatment at all locations.

Conclusion

The GPSkin determines TEWL and SCH accurately in healthy and impaired skin barrier state and can monitor skin barrier function in rosacea during treatment. The GPSkin device is much more practical compared to previous skin barrier tools when used in clinical practice. Its further validation in other inflammatory skin diseases is recommended.

INTRODUCTION

Measurement of stratum corneum hydration (SCH) and transepidermal water loss (TEWL) provide important information about the function of the skin barrier.[1-3] Impaired skin barrier function due to stratum corneum (SC) abnormalities is a hallmark of chronic inflammatory skin diseases, such as atopic dermatitis, psoriasis, and possibly also rosacea.[3-5] SCH and TEWL are also promising markers to distinguish healthy from inflamed skin and to monitor treatment.[6-8]

A variety of skin barrier device methodologies is available to measure SCH and TEWL.[3,9-11] Unfortunately, these conventional devices have various disadvantages; they are expensive, bulky, not wireless, require repeated calibration, and intra- and inter-instrument variation is large, making comparison of study outcomes challenging.[3] For all these reasons, assessment of skin barrier function is currently limited to research facilities with the available financial and logistic resources.

Recently, a new, noninvasive handheld device measuring SCH and TEWL simultaneously was introduced; the GPSkin. It is low-cost, lightweight, pocked-sized, rapid, wireless, and data are directly transmitted to a smartphone application via Bluetooth. Earlier studies showed that the GPSkin provides precise and reliable SCH and TEWL values when compared to conventional devices in healthy skin.[12-14] Moreover, it is able to show skin barrier differences after application of topical agents.[14] However, to our knowledge its validity in case of a damaged skin barrier and its ability to monitor skin barrier function in patients with inflamed skin is not examined yet. As papules and pustules in rosacea often improve during anti-inflammatory treatment,[15] we will use this facial dermatosis as a model to monitor inflamed skin state.

The aim of this study was to determine the value of GPSkin to measure accurate SCH and TEWL values after barrier function impairment, by comparing these values with conventional devices. Moreover, the value of the GPSkin to monitor skin barrier function in daily practice in rosacea patients during anti-inflammatory treatment was determined. To do so, GPSkin values were compared to clinical scores.

MATERIALS AND METHODS

This explorative pilot study, approved by the local medical ethics committee, consisted of two sub-pilots (Fig. 1). In pilot 1, SCH and TEWL were determined with the GPSkin Pro (GPOWER Inc, Seoul, South Korea) at the volar forearm in healthy volunteers before and directly after skin barrier perturbation. As a validation for GPSkin values, parallel measurements with conventional devices were conducted; SCH with the Epsilon (E100, Biox, London, UK), and TEWL with the Aquaflux (AF200, Biox, London, UK). In pilot 2,

GPSkin values were measured at both cheeks in rosacea patients before and during treatment. These values were linked to GPSkin values of healthy controls from pilot 1, and to their clinical symptoms.

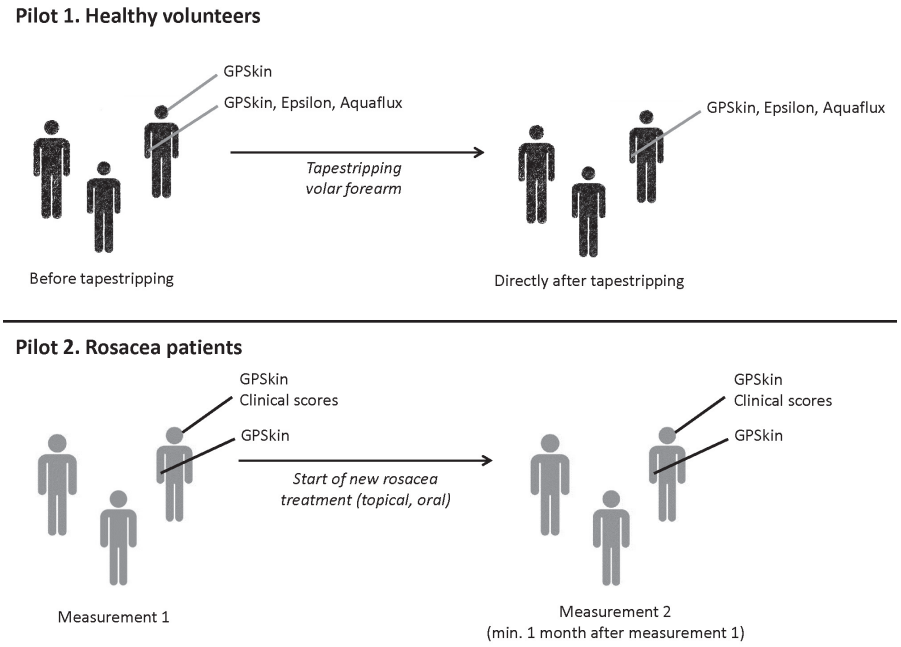

Figure 1. Overview of the study design, consisting of two sub-pilots. **Pilot 1, healthy volunteers**. GPSkin, Epsilon and Aquaflux measurements were performed at the right volar forearm before and directly after tapestripping. Moreover, GPSkin measurements were performed one at the left and right cheek without intervention. **Pilot 2, rosacea patients**. GPSkin values and clinical scores were determined at the left and right cheek before and minimal 1 month after start of new topical and/or oral anti-inflammatory rosacea treatment. Additionally, GPSkin values were determined at both time points at the right volar forearm without intervention.

Devices

GPSkin

The GPSkin measures SCH and TEWL simultaneously by placing its probe onto the skin for 5-10 seconds. For SCH, two electronic sensors at the edge of the probe measure SC capacitance (i.e. dielectric constant). For TEWL, the probe opening (11 x 14 mm) contains a pseudo-closed chamber system with temperature and humidity sensors; this system is similar to a closed chamber system, but provides chamber ventilation to decrease its humidity and pressure. All measurement results are directly sent by Bluetooth to a smartphone application for data access.[12,13,16] The device weights 40 g and is wireless.

Epsilon

Stratum corneum hydration can be measured with the Epsilon, a new generation corneometer, calculating the electrical capacitance of the SC by placing the probe of the device (1.3 x 1.5 cm) onto the skin for 30 seconds. Compared to conventional single sensor corneometers, the Epsilon contains 76 800 sensors, arranged in a 256 x 300 array with a spacial resolution of 50 µm and a sensing depth of 20 µm. Moreover, analysis software is integrated into the device, and linear water-content-based images can be obtained. This allows mapping of SCH and exclusion of regions with poor physical contact between sensor and skin.[17-19] The device is transported in a 2 kg case and measurements require connection to a laptop.

Aquaflux

This device measures TEWL by placing its probe onto the skin for a maximum of 180 seconds. The probe opening (7 x 7 mm) holds a closed chamber equipped with a condenser (-7.65 °C). The condenser acts as a sink for incoming water vapor, crystallizing incoming moisture into ice. Water vapor flux due to diffusion is calculated using the humidity sensor with inbuilt calibration. No recovery time is necessary before starting the next measurement, as the chamber microclimate is controlled, independently of ambient humidity.[10,18,20-22] The device in total weights 1020 g and requires connection to a laptop.

Pilot 1 Healthy skin

Participants

For pilot 1, healthy Caucasian volunteers were included. Informed consent was obtained from each participant. Measurements were performed in August 2019 at the department of Dermatology, Radboud University Medical Center, Nijmegen, the Netherlands. Exclusion criteria were: age < 18 years, diagnosis of inflammatory/acneiform skin diseases, signs of inflammatory/acneiform skin diseases at the measurement sites, and use of immunosuppressive medication. Subjects did not use cream, body lotion or foundation on the day of measurements and refrained from physical activity and showering within 3 hours before the measurements.

Study procedures

The measurements took place at the right volar forearm, because this location is easy to access, mainly refrained from ultraviolet-light damage, hair, and sebaceous glands, and often used as a standard anatomical site for skin barrier studies.[3,22-24] All procedures were performed by one investigator (JGML). A circular area of approx. 3 x 3 cm was demarked with a pen at this location. The demarked skin was acclimatized to ambient air (room temperature: 22-26°C; air humidity: 40-65%) for at least 10 minutes

before start of measurements. Volunteers were placed in upright, sitting position during all study procedures.

First, SCH and TEWL were measured with the GPSkin once at the air-exposed forearm. Then, SCH was determined by performing one measurement with the Epsilon. The *Snapshot* mode was used with a 5 seconds delay after first skin contact, and the average of three frames was calculated automatically. For both devices, moderate pressure was applied to keep contact with the skin surface. Thirdly, TEWL was measured with the Aquaflux. After calibration of this device, two measurements were performed with standard settings and a maximum measurement time of 180 seconds. The average of the two measurements was calculated. The Aquaflux was kept steady and perpendicular to the skin surface with very light skin pressure during measurements.

Next, the skin barrier of the demarked forearm location was disturbed using tapestripping, a noninvasive, painless, widely applied procedure to analyse SC barrier function without interfering with deeper, living epidermal keratinocytes.[9,25-29] Repetitive adhesive tapes were applicated to the skin for 10 seconds with a standardized pressure pen (150 g/cm^2; D'Squame, Monaderm, Monaco) and sequentially removed until the skin became partly to homogeneously refulgent, corresponding to partial to almost complete removal of the SC; 13-33 tapes per volunteer were needed. In this way, a wide range of SCH and TEWL values was obtained.

Directly after the tapestripping procedure, GPSkin, Epsilon, and Aquaflux measurements were repeated at the demarked location as described above. Lastly, one GPSkin measurement per cheek site was performed in each volunteer for later comparison to rosacea patients in pilot 2 (Fig. 1).

Pilot 2 Rosacea

Participants

Patients with a clinical diagnosis of facial rosacea were included in pilot 2 after signing informed consent. They were recruited between July and December 2019 at the department of Dermatology, Radboud University Medical Center, Nijmegen, the Netherlands. Patients needed to start with new topical or oral anti-inflammatory rosacea treatment according to clinical daily practice via their physician.[15] Excluded were patients aged < 18 years, using immunosuppressive medication, or having other facial dermatological conditions or underlying diseases able to interfere with rosacea diagnosis or assessment.

Study procedures

Forearm and facial skin were acclimatized to ambient air for at least 10 minutes before the start of measurements. Then, one GPSkin measurement per cheek site and at the right volar forearm was performed. Additionally, facial clinical assessment was performed including lesion count, investigator's global assessment (IGA), papules and pustules scale, erythema scale, and telangiectasia scale (Table S1, Supplementary Materials). Directly after these measurements, anti-inflammatory rosacea treatment was started; topical ivermectin (n = 13), topical metronidazole (n = 1), doxycycline (n = 1), or topical ivermectin combined with doxycycline (n = 1). Minimally one month later (median follow-up time: 63 days; range: 35-94 days), GPSkin measurements and clinical assessment at both cheeks and the right forearm were repeated.

Statistical analysis

Due to the nonparametric character of data from pilot 1, Spearman correlation analysis (R_s) was used to calculate the relationship between GPSkin values and results obtained from the Aquaflux and Epsilon. Next, a simple linear regression analysis (R^2) was performed to test for a possible linear relationship between the measurements from the GPSkin and conventional devices. For both analyses, values from before and after tapestripping were merged. For pilot 2, differences between baseline GPSkin values of rosacea patients and healthy controls were analysed using the Mann-Whitney U test. A Friedman test with Dunn-Bonferroni post hoc method was performed to demonstrate possible differences between GPSkin results among the three body sites. GPSkin and lesion count differences of rosacea patients at baseline and during therapy were explored with the Wilcoxon signed rank test. For all statistical tests, P-values <0.05 were considered significant. Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, San Diego, CA, USA) and SPSS (SPSS statistics 25, IBM Corporation, USA).

RESULTS

Pilot 1 Healthy skin

Twenty-seven volunteers (18 females and 9 males; median age 37 years, range 23-67 years; skin type I-III) participated in pilot 1. Correlation of GPSkin TEWL and SCH with Aquaflux and Epsilon was very strong (R_s > 0.80; Fig. 2), and also highly linearly related (R^2 > 0.80). Interestingly, the range of Epsilon values was large with GPSkin values ≥ 60. Before tapestripping, median TEWL was 5.1 g/m^2/h (range: 0.4-19.6) for GPSkin and 12.2 g/m^2/h (range: 8.1-17.6) for the Aquaflux. Median SCH was 21 arbitrary units (a.u.; range: 9-38) for the GPSkin and 9.6 a.u. (range: 5.3-16.1) for the Epsilon. After tapestripping, median TEWL was 42.7 (range: 19.6-80.0) for the GPSkin and 70.8 (range: 25.1-88.3) for the Aquaflux. Median SCH was 73 (range: 27-74) for the GPSkin and 45.7 (range: 10.6-63.5) for the Epsilon.

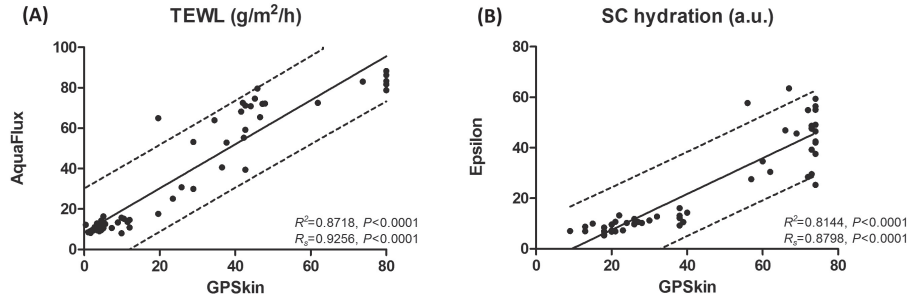

Figure 2. Linear regression with R^2-values and 95% confidence intervals (dotted lines) for GPSkin vs. conventional devices. Spearman correlation coefficient (R_s) is also displayed. GPSkin was tested against the Aquaflux to measure TEWL (**A**), and the Epsilon to measure hydration (**B**).

Pilot 2 Rosacea

Comparison to healthy controls

Sixteen rosacea patients (11 females and five males; median age 51 years, range 21-84 years) participated in pilot 2. No significant differences were found in TEWL GPSkin readings between rosacea patients and controls (Fig. 3A). Post hoc analysis revealed that TEWL at the forearm was significantly lower compared to the left and right cheek, both in rosacea patients as well as controls ($P < 0.05$). SCH was significantly lower in rosacea patients compared to controls at the left and right cheek (Table 1, Fig. 3B). SCH values showed no significant anatomical differences.

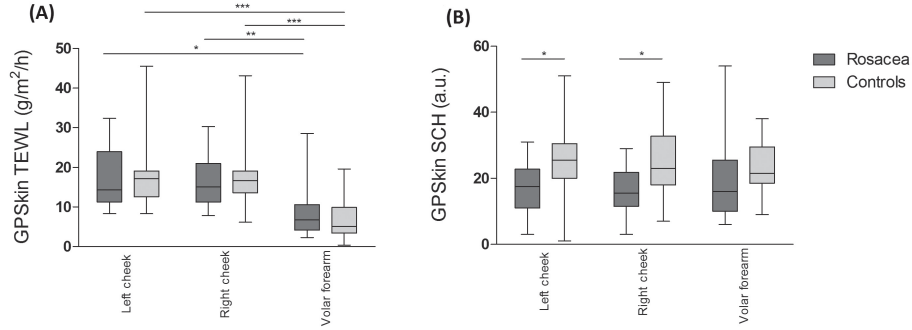

Figure 3. GPSkin results at the left cheek, right cheek, and volar forearm of rosacea patients at baseline and healthy controls. **A**, TEWL, transepidermal water loss. **B**, SCH, stratum corneum hydration. The boxes indicate the median value with 75th percentile and range. * $0.01 \geq P < 0.05$, ** $0.001 \geq P < 0.01$, *** $P < 0.001$

Table 1. GPSkin values of rosacea patients at baseline compared to healthy controls.

	Left cheek			Right cheek			Volar forearm		
	Rosacea (*n* = 16)	Controls (*n* = 27)	*P*-value	Rosacea (*n* = 16)	Controls (*n* = 27)	*P*-value	Rosacea (*n* = 16)	Controls (*n* = 27)	*P*-value
TEWL, *g/m²/h*	14.4 (8.3-32.4)[a]	17.2 (8.3-45.5)	.77	15.1 (7.8-30.3)	16.7 (6.2-43.1)	.91	6.8 (2.3-28.5)	5.1 (0.4-19.6)	.24
SCH, *a.u.*	19 (3-31)	25 (1-51)	**.010**[b]	16 (3-29)	23 (7-49)	**.013**[b]	16 (6-54)	21 (9-38)	.062

[a] Values are expressed as median (range). [b] $P < 0.05$.
a.u., arbitrary unit. SCH, stratum corneum hydration. TEWL, transepidermal water loss

Effect of treatment

All 16 rosacea patients attended the follow-up visit. The GPSkin follow-up data from the volar forearm of one patient were excluded, because TEWL and SCH were very low (4 g/m^2/h and 1 a.u. respectively), probably due to a low battery. Median number of facial inflammatory lesions significantly decreased from 19 (range 0-45) at baseline to 3 (range 0-21; P = 0.001) during treatment. Improvement in IGA and erythema was noticed; telangiectasias remained unaffected (Fig. 4). Compared to baseline, TEWL at both cheeks and the forearm did not change at follow-up (Table 2, Fig. 5). Although not statistically significant, a clear trend towards increased SCH at the left and right cheek was seen during treatment; this increase in SCH was not seen at the forearm. Fig. 6 showed that SCH and TEWL were significantly and negatively correlated (R_s = -0.3970, P = 0.024). No correlations were found between GPSkin values and clinical scores (R^2 all < 0.25; data not shown).

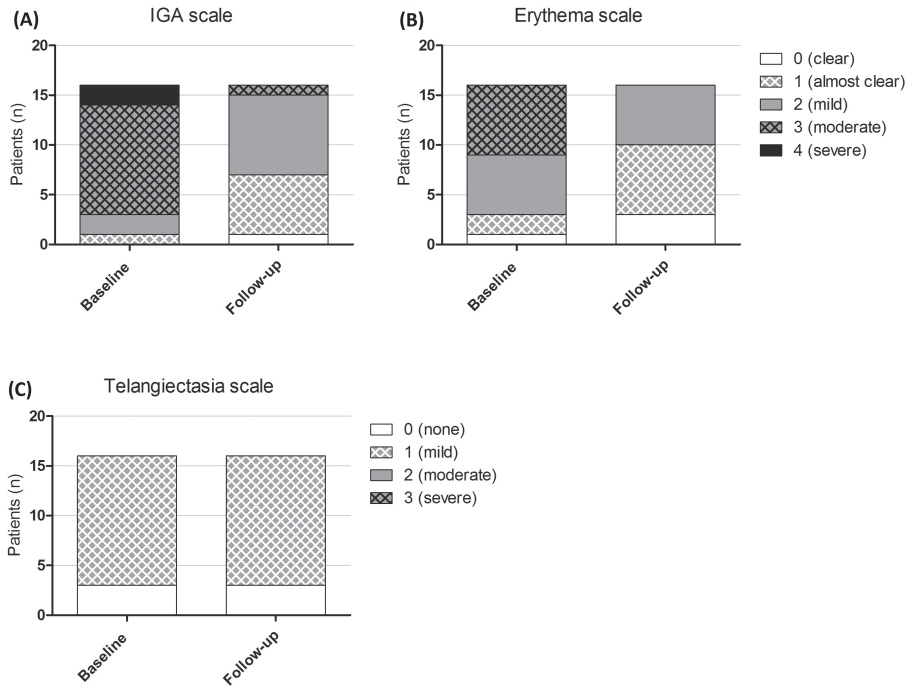

Figure 4. Clinical scores of all rosacea patients (n = 16) at baseline and during treatment (= follow-up). **A-C**, IGA scale, erythema scale, and telangiectasia scale. IGA, investigator's global assessment.

Table 2. GPSkin values of rosacea patients at baseline and during treatment.

	Left cheek			Right cheek			Volar forearm		
	Baseline (*n*=16)	During treatment (*n*=16)	*P*-value	Baseline (*n*=16)	During treatment (*n*=16)	*P*-value	Baseline (*n*=16)	During treatment (*n*=15)	*P*-value
TEWL, $g/m^2/h$	14.4 (8.3-32.4)*	13.6 (6.7-25.3)	.23	15.1 (7.8-30.3)	14.9 (6.7-34.1)	.59	6.8 (2.3-28.5)	7.8 (3.4-28.9)	.36
SCH, *a.u.*	19 (3-31)	22 (13-38)	.11	16 (3-29)	21 (7-34)	.059	16 (6-54)	16 (9-43)	.70

*Values are expressed as median (range).
a.u., arbitrary unit. SCH, stratum corneum hydration. TEWL, transepidermal water loss

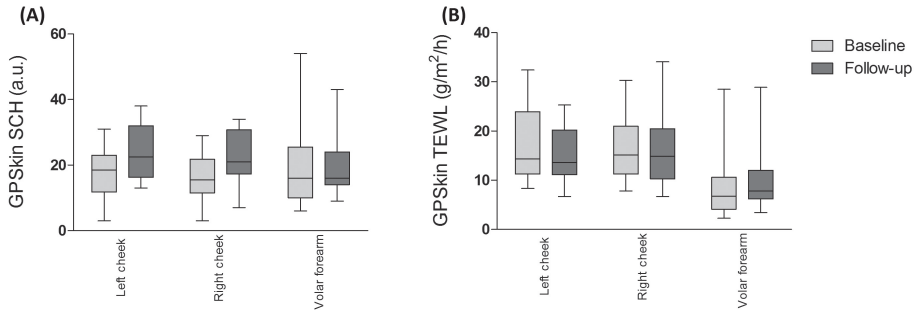

Figure 5. GPSkin results at the left cheek, right cheek, and volar forearm of rosacea patients at baseline and during follow-up. **A**, TEWL, transepidermal water loss. **B**, SCH, stratum corneum hydration. The boxes indicate the median value with 75th percentile and range.

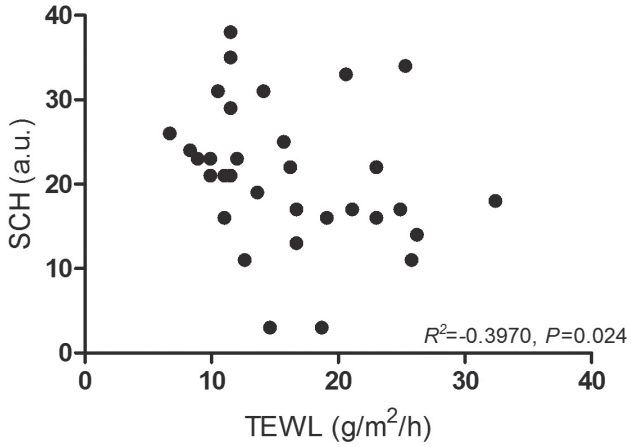

Figure 6. Weak inverse correlation found between stratum corneum hydration and transepidermal water loss in rosacea patients (baseline and follow-up data combined), measured with the GPSkin.

DISCUSSION

We showed that the GPSkin is able to provide reliable and accurate TEWL and SCH values compared to conventional devices, also after skin barrier perturbation. Moreover, we found that SCH, measured with the GPSkin, was significantly lower in rosacea patients compared to controls, with a recovering trend towards normal values after successful rosacea-treatment. TEWL values in rosacea patients were comparable to healthy controls and did not change during treatment.

The GPSkin offers interesting advantages for application in clinical practice compared to conventional skin barrier devices. First, it measures TEWL and SCH simultaneously, preventing precise replacements of probes on the same skin site.[14] Second, data about skin temperature and humidity are displayed. Third, it is extremely portable, affordable, allows rapid and simple measuring, and has a long battery life (months, depending on use frequency), resulting in much higher ease of use compared to the non-wireless, heavier-weighted Epsilon and Aquaflux. Forth, data results are immediately visible at the smartphone screen, allowing immediate feedback to the patient. Based on our experiences within this study, this is very beneficial for improvement of therapy compliance.

In pilot 1, the correlation of TEWL and SCH measured by the GPSkin and conventional tools was very high, both before and after tapestripping. The Aquaflux has a sophisticated chamber system to measure changes in TEWL after tapestripping,[10,20] and the Epsilon provides precise SCH values due to its multi-sensor character.[17] This implicates that the GPSkin is able to provide very accurate skin barrier values as well. Aquaflux TEWL values were consistently higher compared to GPSkin with equal ranges, both before and after tapestripping. This can be explained by calibration differences between both devices.[12] Theoretically, unventilated closed chamber systems such as GPSkin could result in divergent values after prolonged measuring due to water vapor accumulation,[3,10] but this was not observed in this study. Regarding SCH, GPSkin values were higher than Epsilon values. As the Epsilon has multiple sensors, the sensing depth is more superficial compared to conventional devices (which have only one sensor), confining measurement to the 'dry' SC only before tapestripping.[17] After tapestripping, SCH measured by the Epsilon showed larger diversity than those measured by the GPSkin (Fig. 2). This may be caused by tapestripping heterogeneity; this technique often results in skin areas with high damage surrounded by relatively undamaged SC.[10] Due to the relatively large probe surface and multi-sensory character of the Epsilon, all these areas were integrated into the measurement, while GPSkin values were determined based on only one sensor with a smaller probe. Moreover, if the SC is mostly removed, capacitance measurements primarily reflects the hydration state of the 'wet' stratum granulosum rather than the SC;[23,29] especially the Epsilon device seems less reliable in this situation.

The number of tape strips removed varied between the healthy volunteers; we tried to induce a partial to almost complete skin barrier removal, in order to obtain a wide range of SCH and TEWL values. A stronger SC cohesion results in less mass removal, requiring more strips to be applicated for the same barrier disruption effect.[9] Tape stripping procedure may be influenced by contact time, anatomical location, and applied pressure.[30] Therefore, a standardized protocol is needed, which we used.

Current literature is inconclusive regarding potential SCH and TEWL differences in rosacea skin compared to healthy skin, probably due to a large heterogeneity in studied rosacea subtypes, measurement locations, and biophysical devices.[31] We hypothesize that rosacea skin displays decreased SCH due to skin dryness, a frequently mentioned symptom in this skin disease. Application of topical treatment, such as ivermectin, reduces skin dryness and thereby increases SCH. SCH and TEWL were negatively correlated in the rosacea group, implying that an increase in skin hydration could slow down the TEWL.[14] Decreased TEWL after treatment was however not observed in this study. Possibly, skin barrier recovery measured by TEWL takes longer than the immediate moisturizing effect, especially in nonprotected areas such as the face.[14,32,33]

Both in rosacea patients and in controls, TEWL of the forearm was lower than the cheeks. This is in line with previous work; anatomical differences in TEWL may be caused by intrinsic differences in eccrine sweat gland and sebaceous follicle activity, skin temperature, blood flow, SC thickness, lipid content, and corneocyte size and turnover time.[33-36] Most likely, also external physical effects in the face may cause differences in TEWL values. No chiral skin barrier differences seem to occur between the left and right forearm.[36,37] However, regional differences for both TEWL as well as SCH exist within short distances of the face, requiring measurements at exactly the same place during follow-up.[33,36-38] This makes rosacea a challenging model for skin inflammation.

In pilot 2, we deliberately chose not to use a climate room during measurements, as this prevents translation of our results into the daily, clinical setting. We accepted normal fluctuations in weather, season, and daytime. Despite this, TEWL values were constant at baseline and at follow-up, implying that external factors did not have a large impact on the results at both time points. This is a very interesting finding, making application in daily practice certainly feasible. However, it remains important to interpret skin barrier results in the light of potential influencing external factors such as temperature, humidity, occlusion, ultraviolet-light, anatomical location, cream use, physical activity, and sweating.[5,35,39,40] Considering the inter-individual variations in SCH and TEWL, a baseline value should always be registered in each patient, and lesional skin should be compared with non-lesional skin.[9]

CONCLUSION

The GPSkin allows accurate, simple, and rapid determination of TEWL and SCH, both in normal as well as in impaired skin barrier. Moreover, the GPSkin is able to measure improvement in skin barrier parameters in inflamed skin during successful treatment and could therefore possibly contribute to objectification of treatment effectiveness. Based on our results, influence of external factors on GPSkin values seems to be limited. Further validation of the GPSkin in other inflammatory skin diseases with impaired skin barrier, such as atopic dermatitis and psoriasis, is preferred. Ultimately, the GPSkin would replace the conventional, expensive, and relatively complex skin barrier tools, both in research and clinical setting. This paves the way for objective, home-based skin barrier monitoring for patients with a variety of inflammatory skin diseases, further improving patient-centred care and therapy compliance.

SUPPLEMENTARY MATERIALS

Table S1. Clinical scores.

1. Lesion count

Location	Papules (n)	Pustules (n)	Nodules (n)
Forehead			
Chin			
Nose			
Left cheek			
Right cheek			

Papule: circumscribed solid elevation < 1 cm
Pustule: circumscribed elevation with white exsudates < 1 cm
Nodule: circumscribed solid elevation > 1 cm

2. Investigators Global Assessment (IGA) scale (0-1 = success, 2-4 = failure)

Score	Grade	Clinical description
0	Clear	No inflammatory lesions present, no erythema.
1	Almost clear	Very few small papules/pustules, very mild erythema present.
2	Mild	Few small papules/pustules, mild erythema.
3	Moderate	Several small or large papules/pustules, moderate erythema.
4	Severe	Numerous small and/or large pustules, severe erythema.

3. Papules and pustules scale

Score	Grade	Clinical description
0	Clear	Clear skin with no inflammatory (pustules) or noninflammatory (papules) lesions
1	Almost clear	Rare noninflammatory lesions with no more than one small inflammatory lesion
2	Mild	Some noninflammatory lesions with no more than a few inflammatory lesions (papules/pustules only, no nodular lesions)
3	Moderate	Up to many noninflammatory lesions and may have some inflammatory lesions, but no more than one small nodular lesion
4	Severe	Up to many noninflammatory and inflammatory lesions, but no more than a few nodular lesions

4. Erythema scale

Score	Grade	Description of background erythema
0	Clear	No redness present. Background erythema is consistent with non-involved areas.
1	Almost clear	Slight and localized background erythema in involved areas of the face, usually limited to the malar prominence of the cheeks. Gives the impression of a healthy glow to the cheeks.
2	Mild	Slight to mild background erythema NOT limited just to the cheeks, but extends to the lateral cheeks, chin, or forehead.
3	Moderate	Definite background redness, easily recognized, and extending to lateral cheeks, chin, or forehead.
4	Severe	Severe background erythema over the entire face.

5. Telangiectasia scale

Score	Grade	Description of telangiectasia: fine superficial blood vessels that are visible near the surface of the skin
0	None	No telangiectasia present on the face.
1	Mild	Some to a few telangiectasias are present on the face.
2	Moderate	Many telangiectasias, easily recognized, and extending to lateral cheeks, chin, or forehead.
3	Severe	Many to numerous telangiectasias over the entire face that blends in or matches with the erythema caused by inflammatory lesions. Few matted and dense cluster of vessels present.

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CHAPTER 3

Rosacea

CHAPTER 3.1

Noninvasive objective skin measurement methods for rosacea assessment: a systematic review

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ABSTRACT

Background

Rosacea assessment and therapy monitoring can be challenging to standardize, as most clinical evaluation systems are prone to interobserver variability and not always validated. Therefore, objective, reliable and preferably noninvasive measurement tools are needed.

Objectives

To give insight into available noninvasive imaging techniques and biophysical methods in rosacea by performing a systematic review.

Methods

PubMed, Embase, Cochrane and Web of Science databases were searched until 1 September 2018 in accordance with PRISMA guidelines, to identify studies providing original data about objective noninvasive imaging and/or biophysical skin measurement techniques for diagnosis, assessing severity or therapy monitoring of adult patients with cutaneous facial rosacea. Risk of bias of included articles was assessed with the Cochrane Risk of Bias tool, Quality in Prognosis Studies tool, and the Newcastle–Ottawa Scale.

Results

A total of 78 studies were included, describing 14 imaging and biophysical methods. Widespread information about (sub)surface cutaneous morphology and functionality was obtained. Methodological study quality was relatively low and interstudy outcome variability was large. Several tools show promising value in research settings: for treatment follow-up *Demodex* mites are countable with reflectance confocal microscopy, spectrometry can quantify erythema, and rosacea severity could be objectified with skin hydration- and transepidermal water loss measurements.

Conclusions

This systematic review describes the spectrum of noninvasive imaging and biophysical methods in rosacea assessment, giving multifaceted information about structure and properties of rosacea skin, especially useful for research purposes. Larger studies with good methodological quality are needed to create validated protocols for further implementation into research.

INTRODUCTION

Rosacea is a chronic inflammatory skin disease of uncertain pathophysiology; many factors may play a role in disease development.[1-9] Initially, four rosacea subtypes were described: erythematotelangiectatic, papulopustular, phymatous and ocular. Recently, the classification system was changed from subtype-based to phenotype-based to increase diagnostic and presentation accuracy, but as many trials predate the updated phenotype approach, the subtype-based system still dominates rosacea literature.[8,10-12] To achieve optimal results, rosacea treatment is preferably adjusted to clinical symptoms and disease severity.[3,13,14] However, the variety of clinical manifestations among individual patients and presence of various classification systems make standardization and quantification of rosacea measurements challenging.[11,15] Currently, clinical features represent the gold standard to establish the diagnosis.[8,11] Various numerical scales exist to assess erythema, telangiectasia, papules, pustules and global rosacea score for research purposes.[16,17] These scales are subjective and often not validated, decreasing confidence in the validity of reported outcomes.[7,16-19] Moreover, visual evaluation alone cannot appreciate processes unfolding below the skin surface. Histopathological findings are nonspecific,[1] but experienced dermatopathologists are generally able to diagnose rosacea based on characteristic histological and immunohistochemical features,[20] which can overlap among subtypes.[1,15] Recently, a study showed that high *Demodex* densities (measured by two consecutive standardized skin surface biopsies (SSSBs)) were associated with papulopustular rosacea;[21] however, this sampling method can cause slight ephemeral irritation and the test has yet to be confirmed by other independent studies and for other clinical features of rosacea.

Due to these complexities standardized, validated, objective, reliable and preferably noninvasive measurement tools are needed. The advantage of noninvasive over invasive techniques is the ability to monitor the same facial skin location over time without causing irritation, damage or alteration, including inflammatory responses, which may interfere with diagnosis and evaluation. A variety of noninvasive objective skin measurements exist.[22-25] These techniques are widely used in rosacea, but a comprehensive overview is lacking. This systematic review elucidates the spectrum of available noninvasive objective skin measurement techniques for rosacea. The current literature was reviewed for the following purposes:

1. To provide an overview of available noninvasive objective skin measurement techniques for assessing diagnosis, severity and therapy monitoring of rosacea;
2. To assess displayed skin features of these tools, including technique advantages and limitations;
3. To provide recommendations for future use of these tools in an investigational setting.

METHODS

The study protocol was registered in PROSPERO (registration number: CRD42018108401).[26] A systematic literature search following PRISMA guidelines was performed in four electronic databases:[27] PubMed, Embase, Cochrane Library and Web of Science. The search was based on studies using objective noninvasive imaging and/or biophysical skin measurement techniques for diagnosis, assessing severity or therapy follow-up of rosacea. Skin measurement tools were extracted from literature and by exploring their PubMed MeSH-terms.[4,22-25,28] We defined 'noninvasive' as every method that theoretically cannot lead to skin irritation, bleeding or scarring; this excluded biopsies, epilation of eyelashes/hairs, use of tape or glue onto the skin, collection of skin scrapings or excretions from sebaceous follicles. Only studies involving adult patients with cutaneous facial rosacea were included (Table S1, Supplementary Materials).

All databases were searched to include published studies from date of inception until 1 September 2018. Full details on the search strategy are available in Table S2 (Supplementary Materials). Titles and abstracts were screened for relevance by two independent reviewers (JGML and FMCV). Next, full texts were critically assessed for eligibility by the same independent reviewers. Missing full texts were requested via e-mail from study authors and Radboud University Medical Library. In both phases, any differences regarding inclusion between the reviewers were resolved by discussion. Excluded were papers involving patients aged < 18 years; ocular, extrafacial and drug-induced rosacea; therapeutic techniques; subjective measurements; *in vitro* and animal studies; studies in languages other than English, German or Dutch; meta-analysis, (systematic) reviews and abstracts of congresses or with unavailable full texts. The reference lists of all included articles were checked for relevant articles not identified by the initial search.

Extracted study characteristics included study design, number of participants, rosacea type, assessed skin parameters, measurement locations, method type/aim/findings, and use of reference test. A narrative synthesis was conducted for imaging techniques and biophysical methods separately. Risk of bias was assessed by two reviewers (JGML and FMCV), with disagreements resolved by discussion. The Cochrane Risk of Bias tool was used for assessment of risk of bias in randomized controlled trials (RCTs), with studies graded as having low, high, or unclear risk of bias.[29] For case–control studies, the Newcastle–Ottawa Scale was used.[30] For prognostic cohort studies without a control group (including case reports and case series), the Quality in Prognosis Studies (QUIPS) tool was used.[31] For the QUIPS, overall risk of bias for each of the studies was judged as: (1) low, if there was a low risk of bias in all key domains; (2) unclear, if there was an unclear risk of bias for one or more key domains; and (3) high, if there was a high risk of bias for one or more key domains.[32]

RESULTS

A total of 3690 articles were identified (Fig. 1). After removal of duplicates, 2140 articles were assessed for screening. This resulted in inclusion of 260 abstracts, eligible for full-text screening. Finally, 78 articles were included for this systematic review: 36 articles concerned skin imaging techniques and 79 articles were about noninvasive biophysical measurements. Several studies were included in multiple categories, because more than one method was used (Table S3, Supplementary Materials). Most of the included studies in this review were cohort studies (n = 31) and case–control studies (n = 24), followed by RCTs (n = 14), case reports (n = 8) and case series (n = 1). Below and in the table which is available online (link: https://onlinelibrary.wiley.com/doi/abs/10.1111/bjd.18151), all imaging techniques and biophysical skin measurement methods included in this review are presented. Advantages and limitations of each technique are presented in Table 1 (end of chapter).

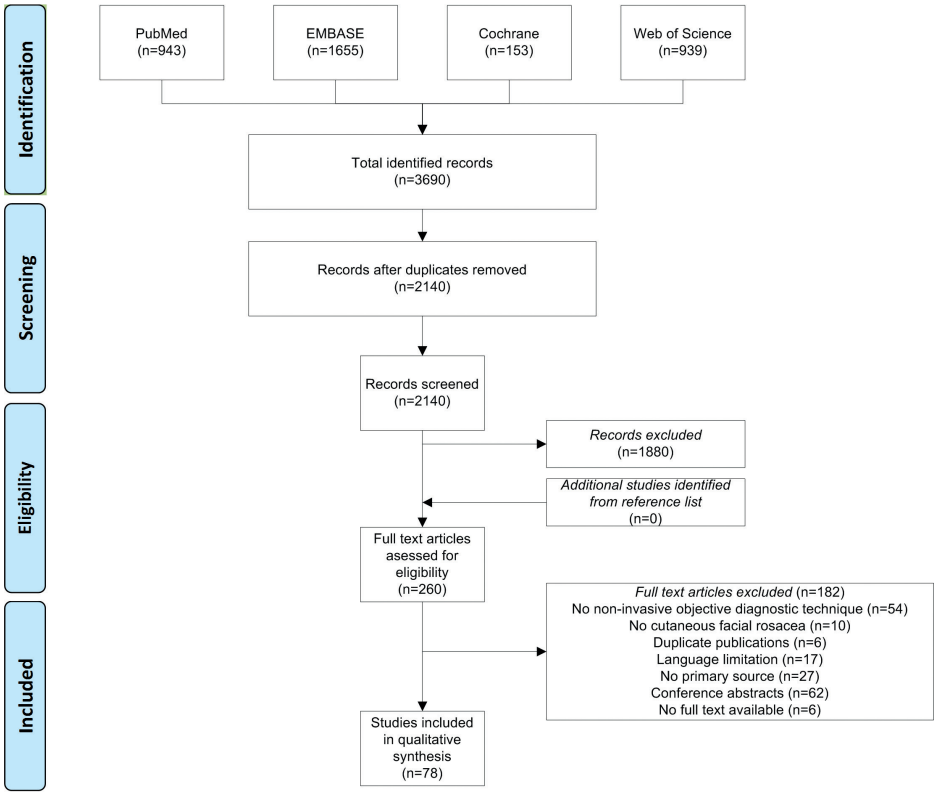

Figure 1. Flow chart: article selection process.

Imaging techniques

Reflectance confocal microscopy

Six studies described use of reflectance confocal microscopy (RCM, also called confocal laser scanning microscopy) in diagnostics and therapy monitoring of rosacea.[33-38] Imaging items of interest were *Demodex* mites, inhabiting human facial sebaceous follicles.[39]

Demodex manifested as roundish/elongated cone-shaped grey structures, surrounded by a bright ring.[33-36] All case–control studies showed significantly higher *Demodex* numbers in patients compared with controls.[33-35,38] There was evidence that patients with papulopustular rosacea (PPR) had significantly higher mite numbers than those with erythematotelangiectatic rosacea (ETR),[35] and that RCM measures higher *Demodex* densities compared with only one SSSB (when two consecutive SSSBs were not taken).[34] Moreover, a significant reduction in *Demodex* mites after topical treatment was seen, correlating with clinical improvement.[36,37] Treatment also resulted in qualitative changes of residual mite appearance.[37] In contrast, Harmelin *et al*. reported *Demodex* disappearance in six of eight treated patients, while complete clinical resolution (not further specified) was established in only three from the six patients with *Demodex* disappearance; the other three showed clinical improvement, but did not resolve completely.[33] No correlation between clinical severity and mite density was found by Falay Gur *et al*.[38] It was not possible to distinguish mite species, viability or life stages.[35,36,38]

Dermoscopy

In total, eight studies used dermoscopy[40-47] (of which two studies used polarized light[40,41]), and one article used video dermoscopy for diagnostic and monitoring purposes.[48] ETR was the most studied rosacea subtype.[42,43,46-48]

Lallas *et al*. showed a 100% presence of vascular polygons in patients with ETR.[42,43] Vascular polygons were also seen in granulomatous rosacea,[45] together with a rosette sign.[41] Unfortunately, rosette signs are nonspecific optical effects of polarized light interacting with keratin-filled adnexal openings, observable in a wide range of skin neoplasms.[41,49] Two studies showed a reduction of background erythema after laser treatment.[44,46] Vascular changes were described in three studies: decrease of vascular network,[47] vessel density[44] and capillary diameter.[48] However, Lallas *et al*. showed that polygonal vessels only disappeared in four of 12 patients after treatment with topical metronidazole, while clinical improvement (not further specified) was seen in eight patients;[43] Micali *et al*. noticed persistence of telangiectasias after brimonidine application while clinical improvement was substantial.[46] Segal *et al*. noticed *Demodex* tails and follicular openings, and reticular dilated vessels in patients with microscopically proven demodicosis using skin scrapings.[40]

Capillaroscopy

Two case–control studies involving capillaroscopy were performed in patients with rosacea.[50,51] Fonseca *et al*. examined the nail-fold capillary beds in eight patients with rosacea and controls with a stereomicroscope.[51] No specific capillaroscopic patterns in rosacea were found. Rosina *et al*. performed videocapillaroscopy on the cheek and nail folds in patients with ETR, seborrheic dermatitis and healthy controls.[50] In the nail folds, this study too did not show any differences between all three mentioned cohorts. On the cheek, patients with ETR had significantly larger polygons, more prominent telangiectasia, larger mean vessel diameter and neoangiogenesis. Moreover, they showed a reddish background due to subpapillary vessel dilation.

Optical coherence tomography

Two studies used optical coherence tomography (OCT) in rosacea for *Demodex* quantification and therapy monitoring.[52,53] Earlier research used OCT to visualize dermal vessels in normal skin.[54-56]

In Maier *et al*., OCT showed *Demodex* mites in 20 patients with rosacea – *Demodex* folliculitis and *Demodex*-aggravated dermatitis perioralis (Table 1).[52] *Demodex* was visible in all patients as bright, round, grouped dots in the superficial part of dark hair follicles, while skin scraping tests were mite-positive in only 15 patients. Mite number was significantly higher in patients than in controls (positive predictive value 67%, sensitivity 100%, specificity 65%). The case report of Urban *et al*. demonstrated decreased dermal hyporeflectivity of patients with ETR after treatment with brimonidine, suggesting a decrease in dermal oedema.[53] No significant changes in vessel diameter were seen.

Computer-aided imaging analysis

Fifteen articles used computer-aided imaging analysis in rosacea diagnosis, severity assessment and therapy monitoring, mainly in ETR.[46,48,57-69] To measure erythema, five studies used the VISIA® Complexion Analysis system (Canfield Scientific, Parsippany, NJ, U.S.A.),[46,62,63,68,69] a commercially available high-resolution facial imaging system with quantitative imaging-analysis software (RBX®; Canfield Scientific), which separates red skin-colour components.[70] In all studies, reduction of concentrated facial dark-red areas after treatment (brimonidine, laser, photodynamic therapy) was seen, corresponding to a reduction in erythema. The remaining 10 articles incorporated a variety of spectral imaging methods (L*a*b* colour space,[60,61,67] red–blue difference index,[61] erythema dose,[61] red–green–blue imaging,[57,59,66] emission of visible and infrared light[48]) to quantify erythema,[57,58,60,61,64-67] telangiectasias[57] and skin haemoglobin distribution.[48,59]

Other techniques

Sonographic imaging and infrared photography are two additional imaging techniques used in rosacea cases.[71,72] Detailed information is summarized in Table 1.

Biophysical methods

Stratum corneum hydration

Skin hydration was assessed in 16 studies by corneometry, mainly for therapy monitoring.[62,66,68,73-85] A corneometer measures electrical capacitance of skin surface, providing insight into stratum corneum barrier function,[28] motivated by earlier studies implying decreased barrier function in patients with rosacea.[1,3]

In general, a wide range of skin capacitance values was reported (16.5–381.6 a.u.). Case–control studies demonstrated that skin hydration in patients with ETR and PPR was significantly lower than in controls.[81,82,84,85] Moreover, Kim *et al*. found significantly lower values in facial areas with increased erythema (measured with a skin colour imaging system) compared with areas having normal skin colour, and in severe compared with mild/moderate rosacea.[66] Significant skin hydration increases were seen after successful treatment (moisturizers, topical metronidazole, oral tetracyclines).[75,77-80,82,83] In two RCTs where patients with ETR and PPR used creams containing silymarin or glycine/chitosan, the placebo groups using vehicle creams without these ingredients also had significantly higher skin hydration after treatment.[78,79] Others noticed no significant changes in skin hydration, while rosacea severity decreased after treatment (various topical agents, photodynamic therapy).[62,68,73,74,76]

Transepidermal water loss

Transepidermal water loss (TEWL) was assessed in 16 studies.[66,73-77,80,81,83-90] TEWL is widely accepted as a reference parameter to investigate epidermal permeability barrier function.[91] Seven studies used open measurement chambers,[75,77,80,83,86-88] two studies unventilated closed measurement chambers,[66,90] and in seven studies the measurement principle was not specified.[73,74,76,81,84,85,89]

In general, a wide range of TEWL values was reported (11.5–35.8 g/m^2/h). Patients showed higher TEWL values compared with controls.[66,84-86,89] However, some studies showed no differences at the nasolabial fold,[81] lateral chin,[86] forehead[85] and nose.[85] Kim *et al*. found significantly higher values in severe compared with mild/moderate rosacea.[66] Significant decreases after treatment with various topical creams were observed and were associated with reduced rosacea severity.[75,80,83,87,88,90] Others showed no TEWL differences after treatment with topical agents, while rosacea severity score decreased.[73,74,76,77]

Sebum

Eleven studies were interested in quantifying sebum in rosacea.[38,68,82,84,85,92-97] Seven studies used a Sebumeter®,[38,68,82,84,85,96,97] five studies used other methods (photometric quantification of cigarette paper,[95] gravimetric absorption[92,93] and chromatography[92,94]). It is important to note that a 1:1 correlation between Sebumeter score and amount of

skin oily material is not present.[98] Moreover, reference values for normal facial sebum levels are scarce.[99]

Most studies found normal sebum levels and skin surface lipid composition in patients compared with controls,[38,82,93,94,97] and no correlation with disease severity[93,94] or rosacea subtype existed.[68] Two case–control studies found lower than normal skin surface lipids in patients with PPR,[84,95] and another higher oil levels in patients with nasal rosacea.[85] A fourth study showed significant differences in lipid composition on the back of patients (Table 1).[92] Two studies noticed no change in sebum levels after treatment (topical photodynamic therapy, oral tetracyclines);[68,82] two did (other sebum composition, decrease of sebum levels).[92,96]

pH

Skin pH was measured with pH-meters in three studies.[82,83,85] Results were inconclusive: one case–control study found significantly higher centrofacial pH values in patients with PPR vs. controls,[82] while the other did not.[85] No changes in pH were found in patients with PPR after use of oral minocycline,[82] while the RCT did show significant decreases after application of tranexamic acid solution compared with vehicle treatment.[83] However, also for facial pH, reference values are scarcely available.[99-102]

Erythema

Twenty-two studies assessed monitoring of facial skin erythema by spectrometry.[48,64,68,78-80,82,83,90,96,103-114] Three spectrometric methods were applied;[28] reflectance spectrophotometry,[3,64,68,78-80,82,96,104,106-108,110,111,113] tristimulus colorimetry[83,90,103,109,112] and diffuse reflectance spectroscopy.[105,114] One study used an optical densitometer.[48] In all studies, erythema values decreased after treatment, corresponding to clinical improvement. However, erythema index and L*a*b* colour space values varied enormously (16.3–1002.77 and 6.9–20.2 a.u., respectively). Method of colour calibration was often not described.

Skin blood flow

In eight studies, cutaneous blood flow was established by laser Doppler velocimetry. Overall, no significant differences in facial blood flow between patients and controls were seen, nor after flushing-trigger tasks.[89,115-117] Only Sibenge *et al.* found higher blood flow in facial flushing-affected areas in patients compared with controls.[118] Interestingly, flux was greater in patients with severe rosacea than with mild rosacea[115,116] and values at PPR-affected skin were significantly higher than unaffected skin.[117] Moreover, a decrease of flux was noticed after treatment with Intense Pulsed Light and acupuncture, in line with clinical improvements.[57,119] However, Wilkin *et al.* found no statistical differences in patients after nadolol treatment.[120]

Skin temperature

Skin temperature was measured in three studies.[117,121,122] This was performed with an infrared thermometer,[117] infrared video camera,[122] and iron–constantan thermocouple junctions.[121] No significant differences between ETR- or PPR-affected areas and nonaffected areas or controls were found.[117] Significant skin temperature rises were seen immediately after laser treatment[122] and alcohol ingestion.[121]

Risk of bias

Sample size of most studies was small (and multiple case series/reports). Most RCTs were unblinded or single-blinded (Fig. S1, Supplementary Materials). Moreover, the method of random sequence generation and allocation concealment was often not described. For case–control studies, selection of controls, ascertainment of exposure, and nonresponse rate were often not described (Table S2, Supplementary Materials). For cohort studies, the domain 'Study Confounding' carried the highest risk of bias (Fig. S2, Supplementary Materials) due to insufficient description of potential confounders (e.g. comedication). Approximately 50% of studies did not describe or insufficiently described study population characteristics (domain 'Study Population') and reasons for/potential impact of subjects lost to follow-up (domain 'Study Attrition').

DISCUSSION

Noninvasive, objective methods are needed for reliable assessment and therapy monitoring in rosacea. We included a large number of publications describing (sub) surface morphology and functionality of rosacea skin. This is especially useful for research purposes (e.g. follow-up of new treatments), but can possibly also contribute to elucidation of its multifactorial pathogenesis.

Unfortunately, the quality of included studies was relatively low and interstudy outcome variability was large; various rosacea subtypes, measurement locations, treatments and biophysical devices were used. A description of measurement sites (involved or uninvolved skin) was not always given. Method standardization and validation was often lacking. Additionally, most methods can only measure one or a few parameters in the very complex environment of rosacea symptoms. Subsequently, the gained information may not be conclusive with a single modality; more than one instrument may be useful to obtain a more complete picture.

Imaging equipment can have high purchase costs and require extensive protocols and trained personnel to obtain accurate and reproducible results. Penetration depth and resolution is limited (RCM, OCT and ultrasonography). Vessel irregularities are difficult to quantify (dermoscopy and capillaroscopy), as there is no standard to measure capillary shape.[50,123] *Demodex* could potentially be confused with other similar structures

(e.g. trichostasis, follicular hyperkeratosis) by dermoscopy; RCM does not impose this limitation, as the mites are easily recognizable with this imaging technique.

Biophysical parameters are strongly influenced by intrinsic and extrinsic factors like age, sex, race, circadian rhythm, season, exercise, and acclimatization period;[22,95,99,100,124-131] therefore data collection is inherently difficult to standardize. For TEWL, open measurement chambers are more vulnerable to environmental influences than are closed chambers;[129,132] condenser closed chambers seem to be the most sensitive TEWL system,[132] but this device type was not used in rosacea. For pH, all three studies used pH900 meters, possibly overestimating true pH due to a small electrode area and short stabilization period of only 3 seconds.[130] For erythema, skin light absorption by components other than haemoglobin (melanin, bilirubin) may influence outcomes[64,105,114] and application of variable skin pressure can change haemoglobin level and skin colour.[67,82] For laser Doppler, absolute values are meaningless without measuring sympathetic flux changes and blood vessel density, which is impossible with noninvasive techniques.[89,115] Control for these factors was often not described or accounted for in the reviewed articles, and comparisons with normal skin were scarce. Additionally, biophysical tools are probe-related; they only cover a small facial area, questioning representativeness of the entire face. Placing the device on the same area of interest in follow-up visits is therefore challenging.[64,76,113] Computer-aided image analysis does not impose these problems as whole face erythema is mapped. Moreover, redness values are obtained without performing skin contact. On the contrary, their analysis methods were often experimental based, and neither standardized nor validated; therefore results were difficult to interpret.

Due to the strict exclusion criteria we applied in this review, all techniques that could possibly lead to skin irritation were excluded, as well as near-noninvasive tools like SSSBs and tapes. The SSSB (with two consecutive SSSBs) is a standardized, reproducible, cheap and simple sampling method to measure *Demodex* density as accurately as RCM.[21,133] Sebutape® (Clinical and Derm, Dallas, TX, U.S.A.) enables assessment of the seborrheic activity of skin pores.[134] Another limitation is that language of published studies was restricted to English, German and Dutch for practical linguistic reasons, which may have resulted in language bias.

Despite the above-mentioned limitations, several tools show promising additional value, especially in research settings; for treatment follow-up, *Demodex* mites can be easily counted by RCM, erythema can be monitored with spectrometry, and rosacea severity can be objectified with additional skin-hydration and TEWL measurements. Evidence-based and validated protocols are needed for long-term application of these tools. We recommend using standardized study environments and comparison of lesional with nonlesional skin. Furthermore, studies with larger samples sizes are needed.

In conclusion, this systematic review provides an overview of the available noninvasive imaging and biophysical tools for diagnosis, severity assessment and therapy monitoring of rosacea. A selection of these tools is promising and provide valuable additional information about the structure and properties of rosacea skin in a manner well beyond that achievable through naked-eye examination; however, firstly adequate and validated protocols are needed for further implementation of these tools in research.

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Table 1. Summary of imaging and biophysical non-invasive methods used in assessing severity and therapy monitoring of rosacea.

	Technique	Measurement principle	Skin features displayed	Advantages	Limitations
Imaging	RCM[33-38]	*En face* imaging of epidermis and superficial dermis at cellular resolution with 830-diode laser	*Demodex* mites (structure; number; optimal visualization depth 10-90 µm)	Rapid (10 min); painless; easy protocol; real time; resolution comparable to histology; medium-sized probes for imaging of recessed body parts; portable (VivaScope 3000)	Expensive; limited penetration depth (up to 250 µm); no *z*-axis imaging; training needed for image interpretation; large device; imaging challenges at anatomical surface irregularities (VivaScope 1500); mm-level motion artefact; no mite species/viability/life stage distinction
	Dermoscopy[40-48]	Optical magnified (usually 10-fold) visualization of colours and microstructures in epidermis and papillary dermis	Background erythema; vascular polygons; *Demodex* tails/follicular openings; rosette sign	Rapid; painless; cheap; real-time; easily applicable *Polarized light*: deeper structures visible (erythema, hair follicle contents, haemoglobin) so possible better to evaluate vascular changes; reduced surface shining; avoidance of pressure artefacts (no fluid immersions needed) *Videodermoscopy*: digital image storage; colour calibration; monitoring	Training needed for image interpretation; interobserver variability; pressure artefacts; no quantitative analysis; rosette sign is unspecific; *Demodex* not distinguishable from similar structures (e.g. trichostasis, follicular hyperkeratosis) *Polarized light*: lower illumination and resolution
	Capillaroscopy[50,51]	Optical magnified visualisation of skin microcirculation until deep dermis	Skin capillary shape and diameter	Rapid; easily repeatable; painless; cheap	Vessel irregularities difficult to quantify (subjective); pressure artefacts
	Computer-aided imaging analysis[46,48,57-67,69]	VISIA® system; multispectral imaging with digital analysis	Erythema; telangiectasias; haemoglobin	Quantification; non-skin contact; portable; facial distribution enabling mapping (detailing concentration and location of chromophores with 50-100 µm resolution)	Expensive; limited resolution; lack of imaging in *z*-axis and information on depth; not real-time; influenced by intrinsic factors (melanin content, physiological blood supply)
	Optical coherence tomography[52,53]	Reflections of infrared light source detected based on low-coherence inferometry until reticular dermis	*Demodex* mites (number); (epi)dermal reflectivity	Rapid (2-5 min); real time; cross-sectional/*en face* imaging comparable with histology; high spatial resolution (3-10 µm^2), compared to RCM deeper penetration depth (up to 1.5 mm) and wider field of view	Expensive; lower contrast and axial resolution than RCM (15 µm); no cellular and subcellular details visible, only architectural changes; decreasing resolution at level of reticular dermis; image localization depends on contact gel amount; reduced image quality in uneven skin (papular eruptions)

Table 2. Continued.

	Technique	Measurement principle	Skin features displayed	Advantages	Limitations
	Ultrasonography[72]	Detection of reflected sound waves	Oedema; nodules	Real-time; painless; widely available; portable; inexpensive; clear visualization of dermis and subcutis	Training needed; low resolution; no visualization of epidermis; no distinction between inflammatory infiltrates/oedema/ tumour/scar tissue (similar echo poor areas); image difficulties in recessed body parts
	Infrared photography[71]	Infrared light	Skin vasculature pattern	Painless; rapid; inexpensive	No differentiation between arterial and venous structures; no quantification
Biophysical	Corneometry[62,66,68,73-85]	Electrical capacitance of skin surface	Hydration of SC and upper part of epidermis	Easy to use; rapid; small probes for measurement in recessed body parts; relatively inexpensive	Influenced by intrinsic and extrinsic factors; small facial area covered
	Evaporimetry[66,73-77,80,81,83-90]	Flux density of water vapour	Transepidermal water loss	Easy to use, rapid; small probes for measurement in recessed body parts	Influenced by intrinsic and extrinsic factors (especially open measurement chambers); small facial area covered
	Sebumetry[38,68,82,84,85,92-97]	Photometric quantification of sebum transparency on skin tapes	Skin sebum levels	Easy to use; rapid; inexpensive; extremely accurate quantification of skin oils	Influenced by intrinsic and extrinsic factors; no 1 : 1 correlation between value and skin oil level; value is oily material-dependent; small facial area covered; reference values scarce
	pH meter[82,83,85]	H^+-ion activity with glass H^+ ion- sensitive electrode	Skin pH	Easy to use; rapid (3 s); no skin occlusion effect	Reference values scarce; small facial area covered; reliability questioned (SC is relatively dry, lipid environment); possible overestimation of values due to too short measurement time
	Spectrometry[48,64,68,78-80,82,83,90,96,103-114]	Reflectance spectrophotometry; tristimulus colorimetry; diffuse reflectance spectroscopy	Skin erythema	Easy to use; rapid; small probes for measurement in recessed body parts; relatively inexpensive	Influenced by intrinsic and extrinsic factors; small facial area covered; method of calibration often not described
	Laser Doppler velocimetry[57,89,115-120]	Doppler shift of moving erythrocytes by laser light; electrical signal is processed proportional to blood perfusion (average penetration depth: 0.2 mm)	Relative changes in skin blood flow	Noncontact; inexpensive; portable; data set comparisons on computer	Small facial area covered; influenced by intrinsic and extrinsic factors; no information about depth; no *z*-axis imaging
	Infrared thermography[117,121,122]	Detection of radiation in long-infrared range of electromagnetic spectrum	Skin temperature	Real-time	Influenced by intrinsic and extrinsic factors; less accurate than contact methods

SC, stratum corneum; RCM, reflectance confocal microscopy

SUPPLEMENTARY MATERIALS

Table S1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Cutaneous facial rosacea (including granulomateous and neurogenic rosacea, rosacea fulminans, rosacea conglobata, rhinophyma, otophyma, gnatophyma, metophyma, morbus Morbihan) • Noninvasive objective imaging/biophysical tools for measuring of severity/follow-up (methods that cannot cause skin irritation, bleeding or scarring; includes applying absorbent paper) • Adults (≥ 18 years) • Randomized controlled trials, non-randomized controlled trials, cohort studies, case series, case reports	• Ocular, extrafacial and drug-induced rosacea • Invasive diagnostic tools (biopsies, superficial skin surface biopsies, epilation of eyelashes/hairs, use of skin tape/glue, collection of skin scrapings or excretions from sebaceous follicles) • Therapeutic tools (e.g. laser therapy for relieve of fibrotic tissue in rhinophyma) • Subjective measurements (e.g. estimation of erythema and inflammation based on clinical photographs) • Children (< 18 years) • In vitro and animal studies • Languages other than English, German or Dutch • Meta-analysis systematic reviews and reviews (no primary source) • Full-text not available • Abstracts of congresses • Duplicates

Table S2. Search strategy.

Pubmed	EMBASE	Cochrane Library	Web of Science
No limitations	No limitations	No limitations	No limitations
("Rosacea"[Mesh] OR rosacea[tiab] OR Rhinophyma[tiab]) AND (("Diagnostic Imaging"[Mesh] OR "Spectrum Analysis"[Mesh] OR "Laser-Doppler Flowmetry"[Mesh] OR assessment tool[tiab] OR Dermoscop*[tiab] OR Dermatoscop*[tiab] OR Photograph* [tiab] OR Microscop*[tiab] OR tomograph*[tiab] OR Laser Doppler*[tiab] OR Fluorescence*[tiab] OR imaging[tiab] OR Measurement*[tiab] OR Monitor*[tiab] OR ((Characteristic*[tiab] OR characterisation[tiab] OR characterization[tiab]) AND skin[tiab]) OR Diagnos*[tiab] OR VISIA[tiab] OR mexameter[tiab] OR video*[tiab] OR videos[tiab] OR spectroscop*[tiab] OR spectrophotometr*[tiab] OR Sonograph*[tiab] OR Ultrasound*[tiab] OR Ultrasonograph*[tiab] OR Videocapillaroscop*[tiab] OR Capillaroscop*[tiab] OR Microscopic Angioscop*[tiab]) OR ("Galvanic Skin Response"[Mesh] OR "Water Loss, Insensible"[Mesh] OR "Sebum"[Mesh] OR "Hydrogen-Ion Concentration"[Mesh] OR Water content[tiab] OR water loss[tiab] OR TEWL[tiab] OR stratum corneum[tiab] OR skin surface[tiab] OR moisture[tiab] OR corneomet*[tiab] OR Permittivity[tiab] OR Permeability[tiab] OR Capacitance[tiab] OR Bioimpedance[tiab] OR Conductance[tiab] OR Aquaflux[tiab] OR Biox[tiab] OR epsilon[tiab] OR barrier[tiab] OR Sebum[tiab] OR fatty acid*[tiab] OR pH [tiab]))	(rosacea/ OR rosacea.ti,ab,kw. OR Rhinophyma.ti,ab,kw.) AND ((exp diagnostic imaging/ OR exp spectroscopy/ OR laser doppler flowmetry/ OR assessment tool. ti,ab,kw. OR Dermoscop*. ti,ab,kw. OR Dermatoscop*. ti,ab,kw. OR Photograph* .ti,ab,kw. OR Microscop*. ti,ab,kw. OR tomograph*. ti,ab,kw. OR Laser Doppler*. ti,ab,kw. OR Fluorescence*. ti,ab,kw. OR imaging.ti,ab,kw. OR Measurement*.ti,ab,kw. OR Monitor*.ti,ab,kw. OR ((Characteristic*.ti,ab,kw. OR characterisation.ti,ab,kw. OR characterization.ti,ab,kw.) AND skin.ti,ab,kw.) OR Diagnos*.ti,ab,kw. OR VISIA. ti,ab,kw. OR mexameter. ti,ab,kw. OR video*.ti,ab,kw. OR videos.ti,ab,kw. OR spectroscop*.ti,ab,kw. OR spectrophotometr*.ti,ab,kw. OR Sonograph*.ti,ab,kw. OR Ultrasound*.ti,ab,kw. OR Ultrasonograph*.ti,ab,kw. OR Videocapillaroscop*.ti,ab,kw. OR Capillaroscop*.ti,ab,kw. OR Microscopic Angioscop*. ti,ab,kw.) OR (electrodermal response/ OR skin water loss/ OR sebum/ OR pH/ OR skin conductance/ or skin lipid/ or skin permeability/ or skin potential/ OR Water content. ti,ab,kw. OR water loss.ti,ab,kw. OR TEWL.ti,ab,kw. OR stratum corneum.ti,ab,kw. OR skin surface.ti,ab,kw. OR moisture. ti,ab,kw. OR corneomet*. ti,ab,kw. OR Permittivity. ti,ab,kw. OR Permeability. ti,ab,kw. OR Capacitance. ti,ab,kw. OR Bioimpedance. ti,ab,kw. OR Conductance. ti,ab,kw. OR Aquaflux.ti,ab,kw. OR Biox.ti,ab,kw. OR epsilon. ti,ab,kw. OR barrier.ti,ab,kw. OR Sebum.ti,ab,kw. OR fatty acid*.ti,ab,kw. OR pH .ti,ab,kw.))	Rosacea (MeSH)	(rosacea OR Rhinophyma) AND (("laser doppler" OR "assessment tool" OR Dermoscop* OR Dermatoscop* OR Photograph* OR Microscop* OR tomograph* OR Laser Doppler* OR Fluorescence* OR imaging OR Measurement* OR Monitor* OR ((Characteristic* OR characterisation OR characterization) AND skin) OR Diagnos* OR VISIA OR mexameter OR video* OR videos OR spectroscop* OR spectrophotometr* OR Sonograph* OR Ultrasound* OR Ultrasonograph* OR Videocapillaroscop* OR Capillaroscop* OR "Microscopic Angioscop*") OR ("skin lipid" or "skin potential" OR "Water content" OR "water loss" OR TEWL OR "stratum corneum" OR "skin surface" OR moisture OR corneomet* OR Permittivity OR Permeability OR Capacitance OR Bioimpedance OR Conductance OR Aquaflux OR Biox OR epsilon OR barrier OR Sebum OR "fatty acid*" OR pH))
Until 28-2-2018: **896 hits**	Until 28-2-2018: **1589 hits**	Until 28-2-2018: **147 hits**	Until 28-2-2018: **891 hits**
29-2 to 1-9-2018: **47 hits**	29-2 to 1-9-2018: **66 hits**	29-2 to 1-9-2018: **6 hits**	29-2 to 1-9-2018: **48 hits**
TOTAL: **943 hits**	TOTAL: **1655 hits**	TOTAL: **153 hits**	TOTAL: **939 hits**

Table S3. Overview of categories of included studies.

Imaging techniques (*n* = 36)*
Reflectance confocal microscopy[33-38]
Dermoscopy[40-48]
Capillaroscopy[50,51]
Optical coherence tomography[52,53]
Computer-aided image analysis[46,48,57-69]
Infrared photography[71]
Sonography[72]
Biophysical parameters (*n* = 79)*
Stratum corneum hydration[62,66,68,73-85]
Transepidermal water loss[66,73-77,80,81,83-90]
Sebum levels[38,68,82,84,85,92-97]
pH[82,83,85]
Erythema[48,64,68,78-80,82,83,90,96,103-114]
Skin blood flow[57,89,115-120]
Skin temperature[117,121,122]

*Some studies were included in multiple categories, because they evaluated more than one imaging/biophysical tool.

Table S4. Review author's judgement about each risk of bias item for each included case-control study (*n* = 24) with Newcastle-Ottawa scale.

	Selection				Comparability		Exposure			Quality score
Author, year	Adequate case definition	Representativeness of cases	Selection of controls	Definition of controls	Comparibility of cases and controls for important factor (sex)	Comparibility of cases and controls for additional factors (age etc)	Exposure ascertainment	Same method of ascertainment for cases and controls	Non-response rate	
Basta-Juzbasíc 1992	★	★	★	★	★	★		★	★	8
Burton 1975	★	★		★				★		4
Darlenski 2013	★	★		★	★	★		★		6
Dirschka 2004	★	★	★	★	★	★		★		7
Drummond 2012 [115]	★	★		★		★		★		5
Drummond 2012 [116]	★	★		★	★	★		★		6
Falay Gur 2018	★	★	★	★	★	★		★		7
Fonseca 2011	★	★		★	★	★		★		6
Foolad 2015	★	★	★	★		★		★		6
Gloor 1974	★	★		★	★	★		★		6
Guzman-Sanchez 2007	★	★		★	★	★	★	★	★	8
Harmelin 2014	★	★		★			★	★	★	6
Helfrich 2015	★	★		★	★	★		★		6
Maier 2012	★	★		★	★	★		★	★	7
Metzler-Wilson 2015	★	★		★	★	★		★	★	7
Ní Raghallaigh 2014	★	★		★	★	★		★		6
Pye 1976	★	★		★		★		★	★	6
Rosina 2006	★	★		★	★	★		★	★	7
Silbenge 1992	★	★		★	★	★		★	★	7
Turgut Erdemir 2014	★	★		★	★	★		★	★	7
Turgut Erdemir 2017	★	★		★	★	★		★	★	7
Wilkin 1980	★			★				★	★	4
Xie 2017	★	★	★	★	★	★		★		7
Zhou 2016	★	★		★		★		★		5

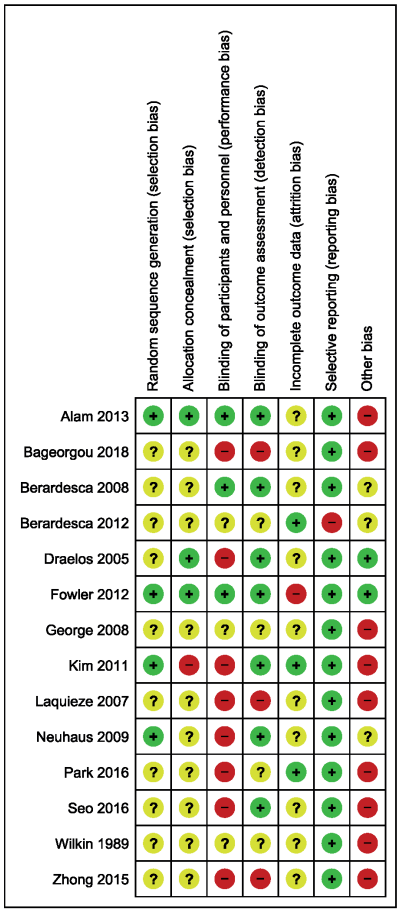

Figure S1A. Review author's judgement about each risk of bias item for each included RCT (*n* = 14) with Cochrane Risk of Bias tool.

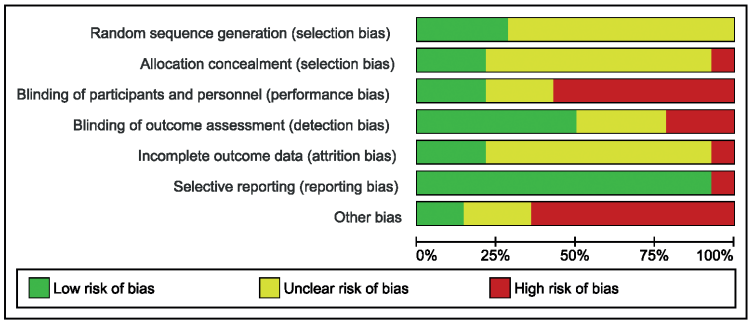

Figure S1B. Review author's judgement about each risk of bias item presented as percentages across all included RCTs (*n* = 14) with Cochrane Risk of Bias tool.

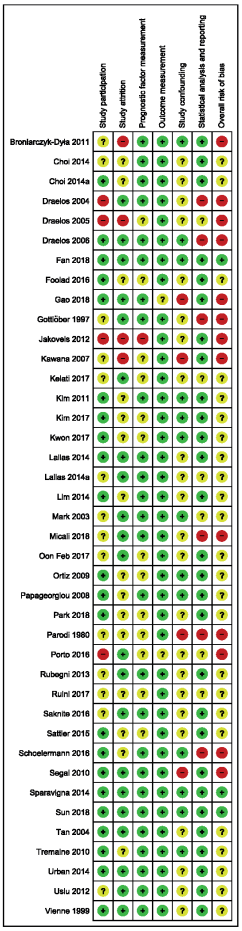

Figure S2A. Review author's judgement about each risk of bias item for each included cohort study and case report/series (*n* = 40) with Quality in Prognosis Studies tool.

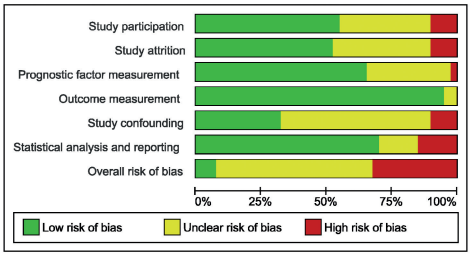

Figure S2B. Review author's judgement about each risk of bias item presented as percentages across all included cohort studies and case reports/series (*n* = 40) with Quality in Prognosis Studies tool.

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CHAPTER 3.2

Value of reflectance confocal microscopy for the monitoring of rosacea during treatment with topical ivermectin

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ABSTRACT

Background

Reflectance confocal microscopy (RCM) enables noninvasive *Demodex* mite detection in rosacea. Objective scoring of rosacea severity is currently lacking.

Objectives

To determine the value of RCM for monitoring *Demodex*, inflammation and vascular parameters in rosacea during treatment.

Methods

In 20 rosacea patients, clinical and RCM examination were performed before, during, and 12 weeks after a 16-week treatment course with topical ivermectin. Using RCM, number of mites and inflammatory cells, epidermal thickness, and vascular density and diameter were measured. RCM features were correlated with clinical assessment.

Results

Treatment resulted in clinical reduction of inflammatory lesions. Mites were detected in 80% of patients at baseline, 30% at week 16, and 63% at week 28. The number of mites reduced significantly during treatment, but no changes in inflammatory cells, epidermal thickness or vascular parameters were observed. Correlation between number of inflammatory lesions and mites was low. None of the RCM variables were significant predictors for clinical success.

Conclusions

RCM enables anti-inflammatory effect monitoring of topical ivermectin by determining mite presence. Quantifying exact mite number, and inflammatory and vascular characteristics is challenging due to device limitations. In its current form, RCM seems of limited value for noninvasive follow-up of rosacea in clinical practice.

INTRODUCTION

Reflectance confocal microscopy (RCM) is a noninvasive imaging technique for *in vivo* visualization of the superficial skin layers with a resolution comparable to conventional histology.[1,2] Unlike invasive tools such as skin biopsies, RCM enables temporal monitoring of the skin without causing irritation, damage or alteration. Historically, RCM has been mainly used for the diagnosis of (non-)melanoma skin cancer,[3-6] but it also has promising potential in the evaluation of inflammatory skin diseases.[7] Some knowledge has already been gained with the detection and quantification of *Demodex* mites in rosacea.[8-13]

Rosacea is a chronic inflammatory skin disorder of uncertain etiology, characterized by among others papules, pustules, erythema, telangiectasia, and flushing.[14-16] *Demodex* mites have been implicated in rosacea etiology,[14,17-19] and studies show that the *Demodex* population is markedly increased in patients with rosacea compared to healthy controls.[20-25] With RCM, mites are visible as roundish or cone-shaped, bright structures inside hair follicles.[8,9,26,27] Moreover, structural changes in *Demodex* mites have been observed after ivermectin treatment using RCM.[28] However, no RCM studies have yet assessed inflammatory and vascular parameters in rosacea.[29,30]

Due to the variety in clinical features among individual rosacea patients and the presence of multiple subjective, often not validated, classification systems, there is a need for methods quantifying rosacea severity in an objective manner.[31-35] It is hypothesized that clinical effects of rosacea treatment can be quantified and ideally also be predicted using RCM monitoring. Topical ivermectin, an antiparasitic preparation that has dual anti-inflammatory and acaricidal effects, has been widely studied as a safe, easy to use, and effective treatment to reduce papules and pustules in rosacea.[36] This makes ivermectin a suitable intervention to monitor rosacea treatment effects.

The aim of this study was to determine the value of RCM to monitor *Demodex* mites, inflammation and vascular parameters in rosacea patients during treatment with topical ivermectin. To do so, RCM features were correlated to clinical scores, and possible prognostic features correlating to successful clinical response were determined.

MATERIALS AND METHODS

Patients

This study was approved by the ethics committee. Patients were recruited between January 2018 and April 2019 at the department of Dermatology, Radboud University Medical Center, Nijmegen, the Netherlands. Eligible participants were ≥ 18 years

old, and had a clinical diagnosis of moderate to severe facial rosacea (investigator's global assessment [IGA] ≥ 3, > 15 papules or pustules, and/or erythema scale ≥ 3 plus telangiectasia scale ≥ 2). Patients currently using ivermectin cream, having other facial dermatological conditions or underlying diseases requiring use of topical or systemic therapies interfering with rosacea diagnosis or assessment, or the inability to adhere to the washout period for these interfering therapies (Table S1, Supplementary Materials) were excluded. Participants were instructed to avoid known rosacea aggravating environmental factors and foods, and to abstain from sunbathing or using tanning beds throughout the study.

Clinical and RCM procedures

Written consent was obtained from all participants. After an acclimatization period of at least 15 minutes, facial clinical scores were assessed including IGA scale, IGA success scale (0 or 1), papules and pustules scale, erythema scale, telangiectasia scale, and inflammatory lesion count (Table S2, Supplementary Materials). Next, participants' cheek with the highest clinical scores (left cheek: $n = 13$; right cheek: $n = 7$) was examined using RCM. The cheek was chosen because highest *Demodex* densities have been found here, and the RCM ring can be attached firmly on the relatively flat surface.[37,38] After examination, participants were instructed to apply topical ivermectin 1% once daily in the evening for 16 consecutive weeks. Clinical and RCM examination were performed at five time points: week 0 (baseline), 6, 12, 16 (during treatment), and week 28 (follow-up). The same cheek was examined with RCM during consecutive visits. Therapy compliance was monitored using patient diaries and by weighing the used ivermectin tubes at week 6, 12, and 16. All examinations were performed in the same room by the same clinician (JGML), and room temperature was kept constant between 19 and 24°C.

RCM device and image analysis

RCM analysis was performed using a Vivascope 1500® confocal microscope (Mavig, Munich, Germany).[39] Four surface-parallel horizontal areas of 8 x 8 mm were scanned with the Vivablock function at the level of the stratum corneum/granulosum, stratum spinosum, dermo-epidermal junction (DEJ), and the superficial dermis. Next, four vertical mappings (Vivastacks) of 0.5 x 0.5 mm were obtained, starting from the skin surface up to a depth of 150 µm with interval steps of 3 µm. Then, three movies of 0.5 x 0.5 mm of approximately 5 seconds each were made in the superficial dermis to visualize dermal blood flow. Analysis of RCM images was performed by one investigator (JGML). Using all Vivablocks, Vivastacks and movies, the number of *Demodex* mites, inflammatory cells, degree of spongiosis, epidermal thickness, and vascular diameter and density were calculated.

Calculating Demodex mites

After selecting one 8 x 8 mm Vivablock at z = 20-80 µm (i.e. stratum granulosum/spinosum; mite quantification is best performed at this level[8,11]), mites were noted as *present* or *absent*. Then, the number of mites, hair follicles, infested follicles, and number of mites per individual follicle were counted; a separate category *doubt* was added if the follicle content was not well defined. Mean number of mites per follicle and mean number of mites per infested follicle were calculated.

Calculating inflammatory cells and spongiosis

The number of inflammatory cells, visible as round-to-polygonal bright cells,[40] was counted in one 8 x 8 mm Vivablock at the level of the DEJ. A group of > 40 inflammatory cells was defined as one inflammatory nest. Dendritic cells were noted as *present* or *absent*.

To determine degree of spongiosis, three single images in a Vivablock with a clear honeycombed pattern from the stratum granulosum were selected. In these images, number of keratinocytes in a square with known surface area without hair follicles were counted manually using ImageJ® freeware,[41] and extrapolated into number of keratinocytes/mm^2. Lower values mean more intercellular edema, indicating increased spongiosis. The mean score of the three images was calculated.

Calculating epidermal thickness

Thickness of the stratum corneum (SC) and the viable epidermis, without SC, were determined (in µm) from the four Vivastacks with a standardized protocol as described in our earlier work.[39] The mean thickness of the SC and the viable epidermis of the four Vivastacks were calculated.

Scoring vascularity

Vascular diameter and density were determined separately from the three movies of the superficial dermis, and classified using a five-point scale: 1 (normal, i.e. similar to healthy facial skin), 2 (mildly increased), 3 (moderately increased), 4 (severely increased), 5 (very severely increased). Example movies of each score can be requested from the author. The mean score of three movies for both parameters were calculated.

Statistical analysis

Descriptive statistics were used to explore baseline characteristics and categorical data. Differences in the same continuous variable between various time points were evaluated with Wilcoxon signed-rank tests for matched pairs. No correction for multiple comparisons was applied, because of the exploratory character of this study. Correlating clinical scores and RCM variables using measurements in the same patients at various time points can lead to biased estimates if the clustering within patients is ignored. Therefore, to get an overall estimate of correlation between

number of inflammatory lesions and number of mites, a meta-analysis was constructed to aggregate the Spearman correlations (*R*) at the five consecutive time points. Additionally, possible interactions between RCM features and achieving IGA 0 or 1 (=clinical success) were measured in term of an odds ratio in the 95% confidence interval by using mixed model logistic regression analysis. Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, Inc., San Diego, CA, USA) and R 3.6.0 (Foundation for Statistical Computing, Vienna, Austria). For all tests, $P < 0.05$ was considered statistically significant. Missing values were excluded from the analyses.

RESULTS

Patient characteristics

Twenty-one patients were included in this study. One patient was excluded because of starting tetracycline during the study. Therefore, data from 20 participants were used for statistical analysis. In one patient, the week 28 visit was not performed due to the patients' inability to travel to the hospital within the study visit window (two weeks). The RCM data of the week 12 visit of one patient were unexpectedly not saved at the computer and were therefore missing.

Demographic and baseline characteristics are displayed in Table 1. Most patients had IGA 3 (80%), erythema 3 (65%), and telangiectasia 2 or 3 (80%) scores. Median facial inflammatory lesion count at baseline was 36.5 (range 0-144). All patients applied ivermectin daily and according to prescription; mean daily quantity applied was 0.36 g.

Eleven patients reported one or more dermatologically related adverse event (AE); stinging/burning (*n* = 7), increased facial erythema (*n* = 4), itching (*n* = 3), and facial skin dryness (*n* = 1). All AEs occurred during the 16-week treatment period and were mild and self-limiting.

Clinical assessment

Fig. 1 presents the clinical scores at the five time points, and representative clinical pictures are shown in Fig. 2. Treatment resulted in clinical success (IGA 0 or 1) in 45% and 47% of patients at week 16 and at week 28, respectively. Median number of facial inflammatory lesions significantly decreased from baseline to week 12 (8.5, range 0-39; $P < 0.001$) and week 16 (2.0, range 0-44; $P < 0.001$), with no further changes at week 28 compared to week 16 (3.0, range 0.0-30; $P = 0.19$). Some improvement in erythema scores was noticed, while telangiectasias remained mainly unaffected.

Table 1. Demographic and baseline clinical characteristics.

Characteristics	Rosacea patients (*n = 20*)
Age, *years*	
Mean ± SD (Min - Max)	52.8 ± 14.5 (24-81)
Gender, *n (%)*	
Female	11 (55%)
Male	9 (45%)
Skin photo type	
I	2 (10%)
II	15 (75%)
III	3 (15%)
Investigator's global assessment	
3 = Moderate	16 (80%)
4 = Severe	4 (20%)
Inflammatory lesion count	
Median (range)	36.5 (0-144)
Erythema scale	
1 = Almost clear	2 (10%)
2 = Mild	3 (15%)
3 = Moderate	13 (65%)
4 = Severe	2 (10%)
Telangiectasia scale	
1 = None	4 (20%)
2 = Mild	8 (40%)
3 = Moderate	8 (40%)

RCM assessment

Demodex mites

Mites were detected in 80% of participants at baseline, 30% at week 12 and 16, and 63% at week 28 (Table 2 and Fig. 3). Median number of mites significantly decreased from baseline (13, range 0.0-203) to week 12 (0.0, range 0.0-10; $P < 0.001$) and week 16 (0.0, range 0.0-14; $P < 0.001$), and remained low at week 28 (1.0, range 0.0-21; $P = 0.51$ compared to week 16). Median number of follicles were comparable before, during, and after treatment. The median number of infested follicles and mites per follicle and mites per infested follicle were all significantly decreased after 16 weeks of treatment ($P < 0.001$, Table 2), while only number of mites per infested follicle increased slightly at follow-up compared to week 16. Most follicles contained zero mites. The percentage of follicles with unclear content (*doubt*) was higher than follicles with clearly visible mites, which is visualized in Fig. 4A-B.

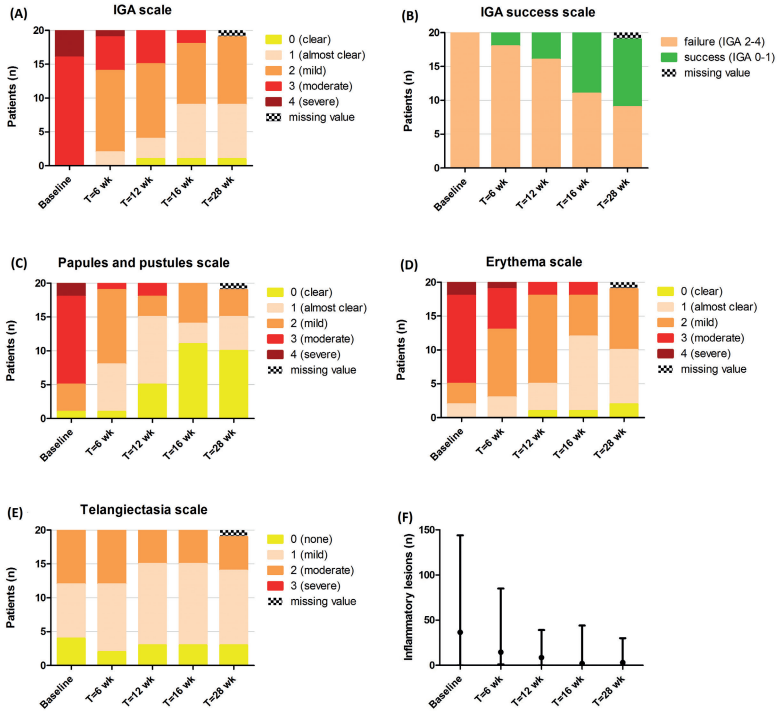

Figure 1. Clinical scores of all patients during 16 weeks of treatment with topical ivermectin and at week 28 (follow-up). **A-E**, IGA scale, IGA success scale (IGA 0 or 1), papules and pustules scale, erythema scale, and telangiectasia scale. **F**, Number of facial inflammatory lesions, expressed as median with range. IGA, investigator's global assessment

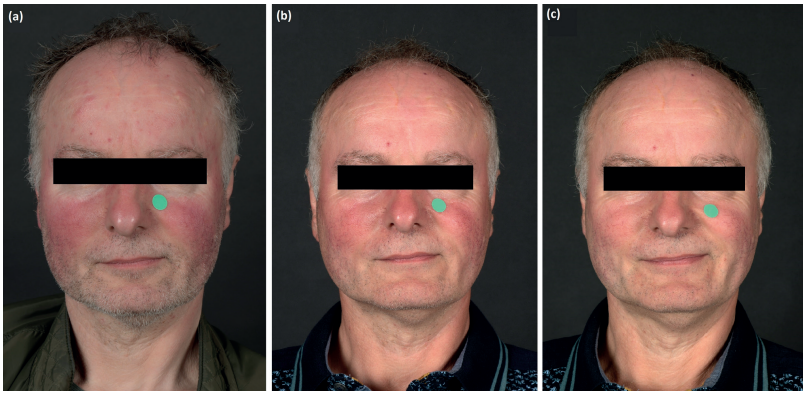

Figure 2. Rosacea in a 56-year old man, **A**, At baseline, **B**, At week 16 of treatment with topical ivermectin 1% once daily, and **C,** 12 weeks post-treatment. Notice the disappearance of papules and pustules at the forehead and prominent erythema at the cheeks, while telangiectasias and residual erythema at both cheeks are persisting. The green dots serve as a tool to assess clinical erythema.

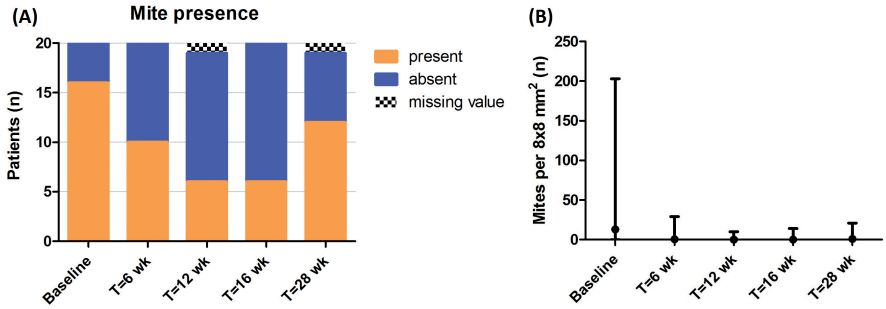

Figure 3. *Demodex* mites during 16 weeks of treatment with topical ivermectin and at week 28 (follow-up), measured with RCM. **A**, Mite presence per 8 x 8 mm, expressed as present or absent. **B**, Number of mites per 8 x 8 mm expressed as median with range.

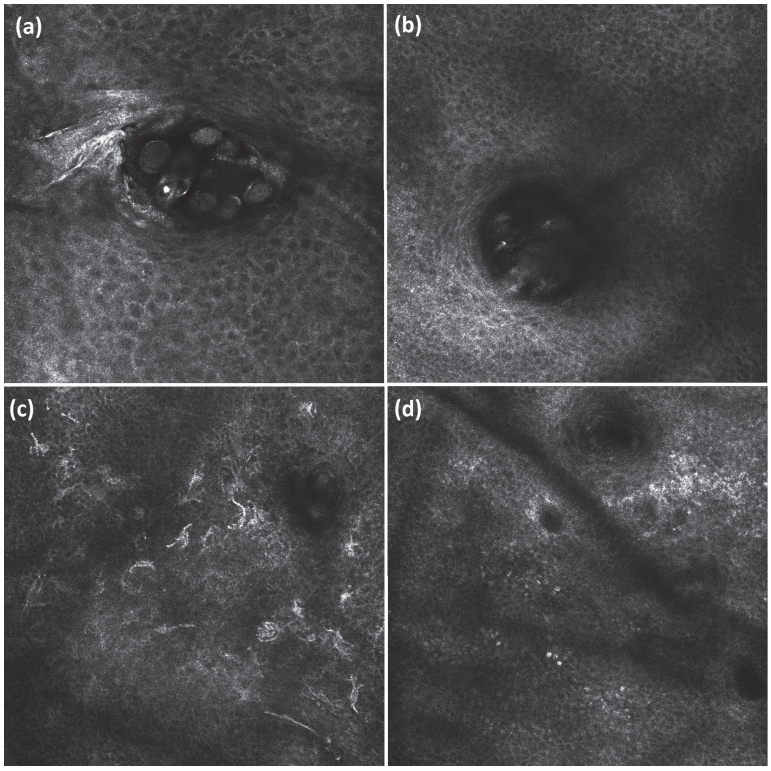

Figure 4. Confocal images of the cheek (0.5 x 0.5 mm). **A**, Follicle at the level of the stratum granulosum containing seven *Demodex* mites, clearly visible as bright, round contours. **B**, Follicle at the level of the stratum granulosum with unclear content due to vague, white-roundish structures, included in mite category *doubt*. **C**, Multiple bright, round to oval or fusiform cells with dendritic processes at the level of the dermo-epidermal junction, possibly melanocytes or Langerhans cells. **D**, Multiple bright, round cells at the level of the dermo-epidermal junction, possibly inflammatory cells.

Table 2. Mite and follicle numbers per 8 x 8 mm.

	Baseline	Week 6	Week 12	Week 16	Week 28	Baseline vs. wk 16 *P*-value	Baseline vs. wk 28 *P*-value	Wk 16 vs. wk 28 *P*-value
Mite presence								
n patients present (%)	16 (80%)	10 (50%)	6 (30%)	6 (30%)	12 (63%)	-	-	-
Mites, *n*	13	0.5	0.0	0.0	1.0	**<.001**[a]	**.001**[a]	.13
Median (range)	(0.0-203)	(0.0-29)	(0.0-10)	(0.0-14)	(0.0-21)			
Follicles, *n*	262	266	263	260	262	.21	>.99	.14
Median (range)	(162-331)	(213-355)	(129-402)	(183-386)	(162-384)			
Infested follicles, *n*	6.0	0.5	0.0	0.0	1.0	**<.001**[a]	**<.001**[a]	.20
Median (range)	(0.0-73)	(0.0-9.0)	(0.0-6.0)	(0.0-6.0)	(0.0-7.0)			
Mites/follicle, *n*	0.052	0.002	0.000	0.000	0.004	**<.001**[a]	**<.001**[a]	.15
Median (range)	(0.00-0.712)	(0.00-0.109)	(0.00-0.039)	(0.00-0.067)	(0.00-0.072)			
Mites/infested follicle, *n*	2.0	0.5	0.0	0.0	1.0	**<.001**[a]	.33	**.036**[a]
Median (range)	(0.0-4.5)	(0.0-4.1)	(0.0-3.0)	(0.0-2.3)	(0.0-4.0)			
Mites/follicle								
Median % of follicles								
0	91	96	97	97	96	-	-	-
1	1.2	0.0	0.0	0.0	0.0	-	-	-
≥2	1.6	0.0	0.0	0.0	0.0	-	-	-
doubt[b]	5.8	3.1	2.8	2.6	3.2	-	-	-

[a] $P < 0.05$. [b] Unclear follicle content, not clearly distinguishable as mites.

Inflammation, epidermal thickness and vascularity

Median number of inflammatory cells, inflammatory nests, and thickness of the SC and viable epidermis did not change significantly at week 16 or at follow-up compared to baseline (Table 3). Dendritic-like cells were seen before, during and after treatment in all patients (Fig. 4C). Interestingly, median number of keratinocytes/mm^2 slightly decreased from baseline to week 16 (P = 0.004) and week 28 (P = 0.031), implying an increase in spongiosis. As shown in Fig. 5, vascular diameter decreased only slightly after topical ivermectin application, while vascular density barely changed.

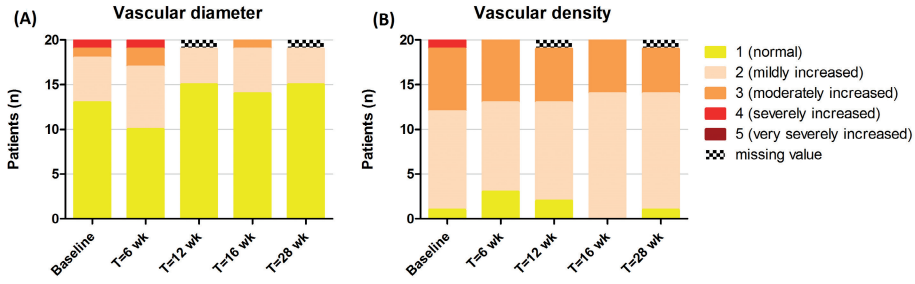

Figure 5. A, Vascular diameter, and **B**, Vascular density scores during 16 weeks of treatment with topical ivermectin and at week 28 (follow-up), determined with RCM.

Correlating clinical scores with RCM parameters

As the correlation estimates in the meta-analysis were homogeneous over time, standard averaging with inversed variance weighting was used in this method. A low correlation existed between number of inflammatory lesions and number of mites (R = 0.28, 95% CI 0.08-0.48). Mixed model logistic regression revealed that none of the RCM variables were significant predictors for clinical success. Despite this, mite presence was the most promising parameter (Table 4).

Table 3. Inflammatory parameters and epidermal thickness.

	Baseline	**Week 6**	**Week 12**	**Week 16**	**Week 28**	**Baseline vs. wk 16 *P*-value**	**Baseline vs. wk 28 *P*-value**	**Wk 16 vs. wk 28 *P*-value**
Inflammatory cells, *n* Median (range)	41 (2.0-494)	31 (0.0-425)	39 (0.0-426)	27 (2.0-370)	51 (1.0-563)	.089	.73	.21
Inflammatory nests, *n* Median (range)	0.0 (0.0-2.0)	0.0 (0.0-4.0)	0.0 (0.0-2.0)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	.17	.089	>.99
Spongiosis, *n keratinocytes/mm²* Median (range)	2519 (1933-3435)	2517 (1668-3186)	2378 (1601-3131)	2268 (1513-2602)	2377 (1723-3108)	**.004**[a]	**.031**[a]	.48
Thickness SC, *µm* Median (range)	10.2 (6.18-18.6)	9.28 (6.18-13.9)	11.6 (6.96-20.6)	10.8 (5.41-27.8)	8.50 (6.18-17.0)	.58	.21	.48
Thickness viable epidermis, *µm* Median (range)	55.3 (44.8-70.9)	54.9 (47.9-70.4)	54.9 (43.3-68.8)	55.7 (44.1-66.5)	54.9 (42.5-67.3)	.32	.30	.51

[a] $P < 0.05$. SC, stratum corneum

Table 4. Mixed model logistic regression models determining RCM features associated with clinical success (IGA 0/1).

	IGA score 0 or 1	*P*-value
Mite presence, *present/absent*	2.10 (0.82-6.07)	.14
Demodex mites, *n*	1.11 (1.01-1.29)	.10
Infested follicles, *n*	1.28 (1.03-1.82)	.088
Mites/follicle, *n*	3.61[12] (55.5-2.49[31])*	.095
Mites/infested follicle, *n*	1.49 (0.97-2.49)	.090
Inflammatory cells, *n*	0.9996 (0.9959-1.0039)	.85
Inflammatory nests, *n*	1.70 (0.65-9.76)	.41
Spongiosis, *n keratinocytes/mm²*	1.0002 (0.9990-1.0017)	.64
SC thickness, *µm*	1.13 (0.94-1.44)	.22
Thickness viable epidermis, *µm*	1.03 (0.95-1.12)	.50
Vascular diameter, *scale 1-5*	0.84 (0.43-1.74)	.61
Vascular density, *scale 1-5*	0.77 (0.33-1.71)	.52

Values are given as odds ratios (95% confidence intervals). IGA, investigator's global assessment.
* Large OR + 95% CI due to low median value and highly skewed distribution of this variable (also see **Table 2**).

DISCUSSION

This study confirmed the well-known efficacy of topical ivermectin to reduce inflammatory lesions in rosacea.[36] To the best of our knowledge, this is the first study combining a broad spectrum of RCM and clinical variables to monitor rosacea during treatment. Using RCM, we were able to monitor the anti-inflammatory effect of topical ivermectin by determination of *Demodex* mite presence, number of infested follicles and number of mites per follicle. However, quantifying exact mite number, inflammatory parameters and vascular characteristics was challenging due to various device limitations.

To guarantee adequate evaluation of rosacea severity, clinical assessment was performed using multiple scales, based on the newly developed phenotype-based classification system for rosacea.[31,36,42] Topical ivermectin treatment reduced the number of facial inflammatory lesions and erythema, with the greatest reduction at week 16. We found no changes in telangiectasias, corresponding to previous results,[36] supporting the hypothesis that telangiectasias are non-inflammatory features. A significant decrease in mite number was measured with RCM, with lowest counts after 12 weeks of treatment, preceding clinical outcomes; these findings suggest that topical ivermectin treatment should be continued for at least 12 weeks. Ivermectin blocks signal transduction in the neuromuscular system of *Demodex* mites, causing paralysis and death.[28,43] Our results support the notion that a reduction of *Demodex* may be causative for the observed decrease in inflammation. Various mechanisms for immune system triggering by *Demodex* have been proposed, such as hair follicle blockage,

foreign-body reactions, and secretion of bacteria and waste.[11,44-46] On the other hand, *Demodex* was absent in 20% of rosacea patients at baseline while they responded well to ivermectin treatment, and correlation between number of inflammatory lesions and mites was low. This suggests that the pathogenesis of rosacea cannot be entirely explained by *Demodex*, and mites could be one of multiple factors inducing follicular inflammation.

Compared to earlier RCM studies calculating *Demodex* density in rosacea,[8,9,11-13,47] we measured low mite numbers, while follicle amounts were very similar.[8,11] These differences could be explained by the fact that we excluded follicles with unclear content from the mite count (Fig. 4B). These follicles could contain sebum, sludge, hair shafts, or (disintegrating) mites.[28,48] Additionally, counting exact mite number was challenging; mites' contours were often vaguely debordered.[49,50] Thirdly, despite the used washout periods (Table S1, Supplementary Materials), we cannot exclude an influence of previous rosacea treatments applied such as topical metronidazole; however, metronidazole does not directly kill *Demodex* mites.[11,51] RCM has some other limitations for mite analysis. First, *Demodex folliculorum* is detectable, but not *Demodex brevis* which lives beneath the RCM maximum visualization depth, namely near the sebaceous glands.[45,50] Second, mite distribution within the follicular canal varies, giving false-negative results when counting mites at only one level.[52] Lastly, RCM does not enable evaluation of mite viability or life stage.[23,49,53,54]

In histological sections, the inflammatory infiltrate in rosacea can be observed in both superficial as well as deeper skin layers.[29,30] In the current study, the DEJ was used as a representative level to score inflammation, because deeper layers impair detailed vision due to reduced image resolution and co-presence of bright collagen bundles.[2] No changes in inflammatory cell number at the DEJ were measured, which may be due to the difficulty of differentiating inflammatory cells from pigmented keratinocytes and melanocytes, which all are visible as polygonal, bright structures (Fig. 4C,D).[2,55] Furthermore, RCM cannot differentiate within leukocyte type.[29,30]

Both vasodilation and increased vascular density are thought to be involved in the pathophysiology of facial erythema in rosacea.[56] We expected to measure a decrease in vasodilation in multiple participants, as this feature is most linked to inflammation, but we did not. Capillary diameter can be easily measured with RCM in vessels inside dermal papillae,[57-60] but as papillae are hardly visible in facial skin,[55] this method is not suitable in rosacea. Therefore, we tried quantifying vascular density and diameter using MATLAB by calculating differences in blood vessel movement between two consecutive movie frames. Unfortunately, these differences were not only caused by blood flux, but also by movement of the vessel surroundings, namely the patient and the RCM device. Consequently, we used Likert scales to grade vascular density and diameter, which are unfortunately still subjective.

Our study has several strengths. All examinations were performed in the same room by the same researcher, reducing light- and interobserver variability. Missing values were minimal due to high visit and therapy compliance. Both genders were equally represented, and age range was large, providing decent generalizability of our findings.

The advantage of RCM is its noninvasiveness without causing discomfort or damage, providing the ability to monitor the same facial location over time.[27] Limitations of our study are due to RCM limitations. Imaging of convex areas is challenging, impeding the generation of a perfectly horizontal Vivablock necessary for analysis in one skin level; image analysis takes time and requires an experienced investigator; RCM images are two-dimensional and in black-and-white only; and the apparatus is bulky, not wireless, and requires start-up of approximately 10 minutes. Taking these factors into account, the RCM in its current form seems unsuitable for quick monitoring of rosacea patients in daily clinical practice. On the other hand, in research setting, RCM provides valuable additional information about mite presence that could otherwise not have been obtained in a noninvasive way.

In summary, we showed that RCM can be used for monitoring of *Demodex* presence in rosacea patients during successful anti-inflammatory treatment with topical ivermectin. Quantifying exact mite number and inflammatory and vascular parameters is challenging due to various device limitations. In its current form, RCM seems of limited value for noninvasive follow-up of rosacea treatment in daily clinical practice. However, as imaging techniques become more compact, intelligent (i.e. enabling automatic counting), and affordable, RCM's future widespread application in research and possibly also clinical practice for rosacea monitoring can be expected.

SUPPLEMENTARY MATERIALS

Table S1. Washout periods for previous topical and/or systemic treatments.

	Treatment	Washout period
Topical	Antibiotics	2 weeks
	Antimicrobial soaps	2 weeks
	Corticosteroids	2 weeks
	Rosacea treatments (azelaic acid, metronidazole, brimonidine)	2 weeks
	Other anti-inflammatory treatments	2 weeks
	Retinoids	4 weeks
Systemic	Antibiotics	4 weeks
	Corticosteroids	4 weeks
	Retinoids	12 weeks

Table S2. Clinical scores.

1. Lesion count

Location	Papules (n)	Pustules (n)	Nodules (n)
Forehead			
Chin			
Nose			
Left cheek			
Right cheek			

Papule: circumscribed solid elevation < 1 cm
Pustule: circumscribed elevation with white exsudates < 1 cm
Nodule: circumscribed solid elevation > 1 cm

2. Investigators Global Assessment (IGA) scale (0-1=success, 2-4=failure)

Score	Grade	Clinical description
0	Clear	No inflammatory lesions present, no erythema.
1	Almost clear	Very few small papules/pustules, very mild erythema present.
2	Mild	Few small papules/pustules, mild erythema.
3	Moderate	Several small or large papules/pustules, moderate erythema.
4	Severe	Numerous small and/or large pustules, severe erythema.

3. Papules and pustules scale

Score	Grade	Clinical description
0	Clear	Clear skin with no inflammatory (pustules) or noninflammatory (papules) lesions
1	Almost clear	Rare noninflammatory lesions with no more than one small inflammatory lesion
2	Mild	Some noninflammatory lesions with no more than a few inflammatory lesions (papules/pustules only, no nodular lesions)
3	Moderate	Up to many noninflammatory lesions and may have some inflammatory lesions, but no more than one small nodular lesion
4	Severe	Up to many noninflammatory and inflammatory lesions, but no more than a few nodular lesions

4. Erythema scale

Score	Grade	Description of background erythema
0	Clear	No redness present. Background erythema is consistent with non-involved areas.
1	Almost clear	Slight and localized background erythema in involved areas of the face, usually limited to the malar prominence of the cheeks. Gives the impression of a healthy glow to the cheeks.
2	Mild	Slight to mild background erythema NOT limited just to the cheeks, but extends to the lateral cheeks, chin, or forehead.
3	Moderate	Definite background redness, easily recognized, and extending to lateral cheeks, chin, or forehead.
4	Severe	Severe background erythema over the entire face.

5. Telangiectasia scale

Score	Grade	Description of telangiectasia: fine superficial blood vessels that are visible near the surface of the skin
0	None	No telangiectasia present on the face.
1	Mild	Some to a few telangiectasias are present on the face.
2	Moderate	Many telangiectasias, easily recognized, and extending to lateral cheeks, chin, or forehead.
3	Severe	Many to numerous telangiectasias over the entire face that blends in or matches with the erythema caused by inflammatory lesions. Few matted and dense cluster of vessels present.

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CHAPTER 3.3

Evaluation of a simple image-based tool to quantify facial erythema in rosacea during treatment

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ABSTRACT

Background

Facial erythema is a common symptom in rosacea. To overcome subjectivity in scoring erythema severity, objective redness quantification is desirable. This study evaluated an image-based erythema quantification tool to monitor facial erythema in rosacea patients during treatment and compared these values to clinical scores.

Materials and Methods

Twenty-one rosacea patients were treated with topical ivermectin for 16 weeks. Clinical erythema scores and clinical photographs were taken at week 0, 6, 16, and 28. Using ImageJ, RGB images were split into red, green and blue channels to measure the green/red ratio of lesional skin compared to a green sticker. With CIELAB colour space, a^* (indicating colour from green to red) of a lesional and non-lesional facial site was measured, calculating Δa^*. Interobserver concordance and correlation between quantitative and clinical erythema values were determined.

Results

Treatment resulted in reduction of clinical erythema scores. No significant changes in red/green ratios were measured. Lesional a^* and Δa^* significantly decreased from baseline to week 16 and 28 ($P < 0.05$). A weak correlation existed between clinical scores and lesional a^* ($R_s = 0.37$), and between clinical scores and Δa^* ($R_s = 0.30$), with a clear trend towards higher a^* and Δa^* for higher clinical scores. Interobserver correlation was high ($R^2 = 0.82$).

Conclusion

ImageJ is a simple, rapid, objective, and reproducible tool to monitor erythema in rosacea patients during treatment. The photographs allow retrospective analysis, evaluation of large and small lesions, and discrimination of subtle redness differences. We recommend using lesional a^* to monitor erythema of inflammatory dermatoses in clinical practice.

INTRODUCTION

Rosacea is a common inflammatory skin disease, often accompanied by facial erythema.[1] Erythema is visible due to increased haemoglobin in the papillary dermis, caused by inflammation, vasodilation and vasculature changes.[2] To achieve optimal results, rosacea treatment is preferably adjusted to clinical symptoms and disease severity. Unfortunately, evaluation of facial erythema by visual assessment lacks objectivity and precision, and is prone to inter- and intra-observer variability.[3-6]

To overcome subjectivity, an objective, noninvasive technique for the measurement of skin colour is desirable. Various noninvasive techniques have already been used to quantify redness in rosacea, e.g. spectrophotometry and computer-aided image analysis (CAIA).[7] Nevertheless, they have some limitations. With spectrometry, erythema is measured in only one point, questioning representativeness of the entire face. Moreover, spectrophotometers require skin contact, changing skin colour due to skin pressure application.[8-10] For CAIA, analysis protocols often included multi-step, complex, time-consuming approaches with expensive and extensive software, or protocols are poorly described, not validated nor standardized,[2,11-19] and therefore difficult to reproduce or use in clinical practice. Additionally, VISIA, a commercially available system with quantitative facial imaging analysis software, does not enable point/segmented erythema analysis, imposing difficulties in areas with diffuse erythema.[2] Lastly, two different colour space methods have been applied in previous studies, namely RGB and CIELAB. RGB represents object "appearance", but does not correct for brightness; CIELAB indicates colour perception, and has the advantage of correcting for variations in brightness.[10,20] Due to these various limitations for erythema quantification in rosacea, a reliable, rapid, non-contact, and simple erythema quantification tool is needed.

The aim of this study was to test an easy-to-use, image-based software tool to quantify and monitor facial erythema in rosacea patients during treatment with topical ivermectin. Additionally, quantified erythema values were correlated to clinical scores and interrater concordance was determined.

MATERIAL AND METHODS

Study participants

Twenty-one patients (9 males, 12 females; skin type I-III; median age 49 years; range 24-81 years) participated in this study. They were recruited between January 2018 and April 2019 at the Department of Dermatology, Radboudumc, Nijmegen, the Netherlands. Subjects were included if they had moderate-to-severe rosacea, defined as an Investigator's Global Assessment (IGA) score of 3 or 4. Patients currently using

ivermectin cream or having other facial dermatological conditions able to interfere with rosacea diagnosis or assessment were excluded. They were instructed to avoid known offending environmental factors and foods triggering rosacea, and not to sunbathe or to use a tanning bed throughout the study.

Treatment, procedures and photography setup

Treatment consisted of topical ivermectin 1% once daily during 16 consecutive weeks. Ivermectin is a potent and easy-to-use anti-inflammatory/acaricidal agent for rosacea with little side effects, making this a suitable intervention to monitor erythema.[21] Clinical erythema was graded using an erythema scale from 0 to 4 (Table 1) at week 0, 6, 16, and 28 (follow-up). During these visits, high-resolution facial photographs were acquired in JPG-format with a commercially available single-lens reflex digital camera (Nikon), equipped with a complementary metal-oxide semiconductor (CMOS) sensor and an AF S Micro Nikkor 105 mm 2.8 objective. All photos were taken under the same light conditions in a photo studio, with a green circular sticker (0.5 inch diameter) attached at the cheek. The camera was manually hold perpendicular to the skin and the sticker, at a distance sufficient to image both the erythematous areas as well as the sticker. Two Broncolor monolights were used to maintain absolute light consistency with respect to exposure and colour. The following settings were used to take photographs: manual focus and mode (M), with aperture and shutting speed adjusted to match optimal exposure; ISO 200; image quality "JEPfine S", corresponding to images with a low (1:4) compression ratio; and colour space sRGB.

Table 1. Clinical erythema severity assessment.

Score	Grade	Description of erythema
0	Clear	No redness present. Erythema is consistent with non-involved areas.
1	Almost clear	Slight and localized erythema in involved areas of the face, usually limited to the malar prominence of the cheeks. Gives the impression of a healthy glow to the cheeks.
2	Mild	Slight to mild erythema NOT limited just to the cheeks, but extends to the lateral cheeks, chin, or forehead.
3	Moderate	Definite background redness, easily recognized, and extending to lateral cheeks, chin, or forehead.
4	Severe	Severe erythema over the entire face.

Erythema quantification

Photographs were analysed with ImageJ® freeware (http://imagej.nih.gov/ij). First, the RGB colour split function was used to divide the original RGB photographs into their constituent red, green and blue channels. The mean *green* intensity of the green sticker was measured. Next, a region of interest (ROI) with the most intense visible facial erythema (i.e. lesional skin) was selected; this was the cheek (n = 19), the forehead (n = 1) or the chin (n = 1). The mean *red* intensity of this ROI was measured. Then,

the mean intensity of the sticker and the ROI were used to calculate the red/green (R/G) ratio as a standardized measure for skin redness. Secondly, the RGB image was converted to CIE *L*a*b** colour space. *L** indicates light intensity from 0 (black) to 100 (white), while *a** indicates colour from green (-60) to red (+60), and *b** indicates colour from blue (-60) to yellow (+60).[9,22] The mean *a** value of the stored ROI was measured, and compared to a representative non-lesional site (neck), serving as a control site for background erythema; *Δa** was calculated (*a** of lesional skin minus *a** of non-lesional skin). Incidental regions of specular reflection were avoided when selecting areas for analysis. A step-by-step guideline for the entire procedure is found in Table 2. Analyses of week 0 and 28 were performed by two independent researchers (JGML and PEJE) to determine interobserver variation; week 6 and 16 analyses were performed by one researcher (JGML).

Statistical analysis

Differences in R/G ratio, *a** and *Δa** between the various time points were evaluated with Wilcoxon singed-rank tests. No correlation for multiple comparisons were applied, because of the exploratory character of this study. Differences in *a** between lesional and non-lesional skin per visit and for both researchers, were explored using Mann-Whitney *U* tests. To test for a possible relationship between clinical and quantified erythema results, Spearman rank correlation (R_s) was used. Lastly, linear regression analysis was applied to determine interobserver variation. Statistical analysis was performed using Instant Clue Software.[23] For all tests, $P < 0.05$ was considered statistically significant. Missing values were excluded from the analyses.

Table 2. Step-by-step protocol for erythema quantification using ImageJ, used in this study.

Original JPG image

1. Drag original JPG photograph into ImageJ *(Photo 1).*
   Split image into the three RGB channels (red, green, blue): **Image → Color → Split Channels.**
   Close blue image, you do not need this one.

RGB green image

2. In *green* image *(Photo 2):*
   Zoom in on green sticker at the cheek.
   Draw a circle inside the green sticker with **'Freehand selections'**.
   Analyse → Measure: record mean green value for green sticker.
   Close green image.

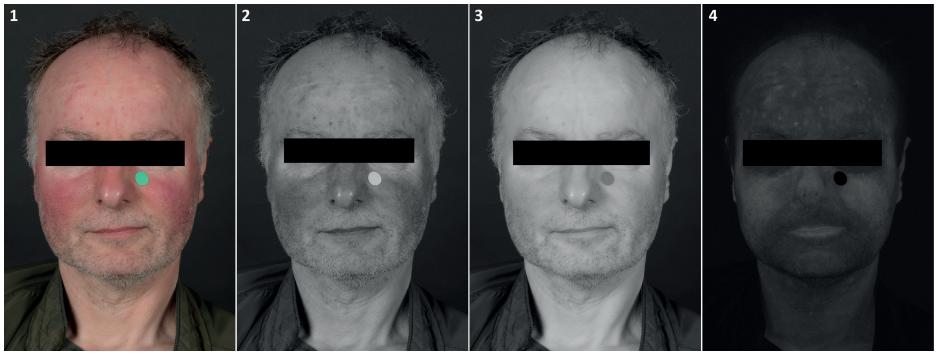
RGB red image

3. In the *red* image *(Photo 3):*
   Choose region of interest of lesional skin. Outline ROI with **'Freehand selections'**. Make sure to avoid any skin colour inconsistency due to pen lines, hair, tattoos, jewellery etc. inside ROI.
   Analyse → Measure: record mean red value.
   Save ROI: **Analyse → Tools → ROI manager.** Click **Add [t] → More → Save.**
   Close red image.

CIELAB a* image

4. In *original* image *(Photo 1):*
   Split image into CIELAB colour space: **Image → Color → RGB to CIELAB.**
   Scroll to second image (*a*, Photo 4).*
   Drag saved ROI into ImageJ; ROI is placed into the *a** image.
   Analyse → Measure: record mean *a** of lesional skin.
   Choose a region with non-lesional skin (e.g. the neck), outline this region with **'Freehand selections'.**
   Analyse → Measure: record mean *a** of non-lesional skin.

5. Transfer all recorded values to a digital database for data analysis.



RESULTS

Clinical scores

Fig. 1 presents the clinical scores. At baseline, 71% of patients had an erythema score of 3 or 4, decreasing to 33% at week 6, 10% at week 16, and 0% at week 28. Only 10% of patients reached an erythema score of 0 at week 28, compared to 0% at baseline.

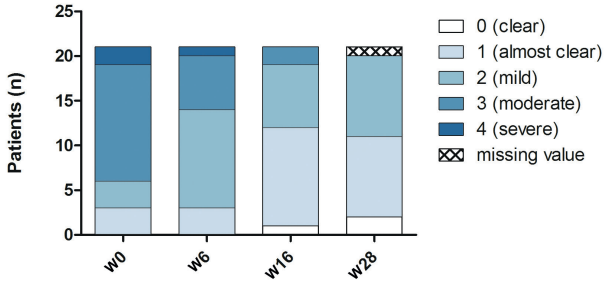


Figure 1. Clinical erythema scores of the rosacea patients at week 0, 6, 16 (during treatment), and 28 (follow-up).

R/G ratio and *a**

Surprisingly, we found no significant changes in R/G ratios during the study (Fig. 2). A significant decrease in median lesional *a** was measured from baseline (24.97, range 19.94-32.95) to week 16 (20.98, range 18.12-34.92; $P = 0.005$) and week 28 (20.68, range 15.17-29.46; $P < 0.001$). No significant differences in non-lesional *a** values were seen during the study, see Fig. 3A. The *a** was significantly higher in lesional skin compared to non-lesional skin at all time points ($P < 0.001$). Δa^* also significantly decreased from baseline (12.23, range 5.52-19.56) to week 16 (9.18, range 1.61-15.40; $P = 0.001$) and week 28 (7.97, range 3.17-16.13; $P = 0.002$), see Fig. 3B.

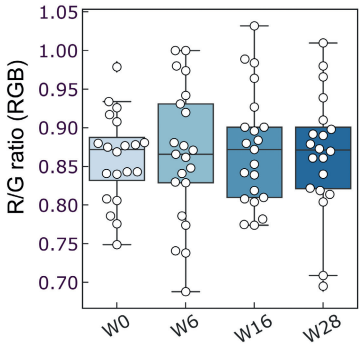


Figure 2. Red/green ratios of lesional skin in rosacea patients per visit. The green values were assessed using a green sticker attached to the cheek. The black lines indicate the median value; the white dots indicate individual values.

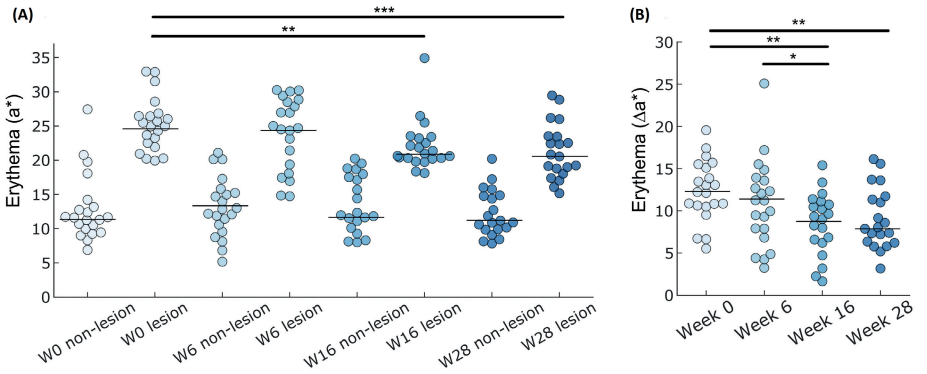


Figure 3. A, *a** values of lesional and non-lesional facial skin in rosacea patients per visit. **B**, *Δa** values (lesional skin minus non-lesional skin) per visit. The black lines indicate the median value. * 0.01 ≥ *P* <0.05, ** 0.001 ≥ *P* <0.01, *** *P* < 0.001.

Correlation of quantified vs. clinical erythema values

A weak correlation was found between clinical erythema scores and lesional *a** (R_s = 0.37, *P* < 0.001; Fig. 4A), and between clinical erythema scores and *Δa** (R_s = 0.30, *P* = 0.007; Fig. 4B). Despite this, a clear trend towards higher *a** and *Δa** for higher clinical scores was visible.

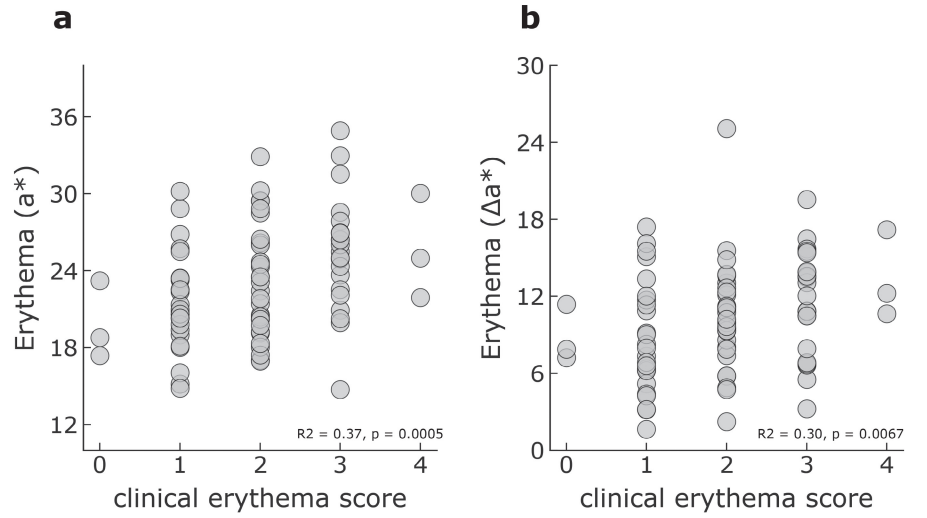


Figure 4. Spearman correlation analysis of clinical erythema scores vs. quantified erythema values. Data of all visits were merged. **A**, Clinical scores vs. lesional *a** values. **B**, Clinical scores vs. *Δa** values (= lesional skin minus non-lesional skin).

Interobserver concordance

Interobserver correlation was high. No significant differences in *a** were found between the two researchers (Fig. 5A), and linear relationship was strong (R^2 = 0.82, P < 0.001; Fig. 5B).

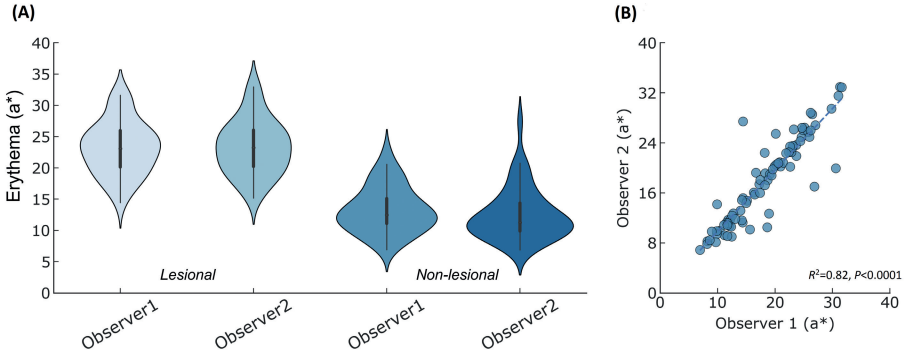


Figure 5. Interobserver variation of *a** at week 0 and 28. **A**, Lesional and non-lesional *a** values of both observers are displayed separately in a violin plot (median, interquartile range, upper and lower adjacent values; density plot width corresponds to frequency). **B**, *a** of lesional and non-lesional skin of both observers are merged for the linear regression analysis.

DISCUSSION

In this study, we evaluated ImageJ, a simple image-based software tool, for the quantification and objective monitoring of facial erythema in rosacea during treatment, and we compared these values to clinical scores. Lesional *a** and *Δa** decreased significantly during treatment, corresponding to a reduction in clinical erythema. The interobserver concordance of *a** was high. R/G ratios did not change during the study and seem unsuitable to monitor redness. Our method is rapid, simple, objective and reproducible; the photographs allow retrospective analysis, evaluation of large and small lesions, and discrimination of subtle redness differences. We recommend using lesional *a** to monitor erythema in daily clinical practice.

Visual erythema assessment, which is currently frequently applied in daily practice for redness monitoring in rosacea, has some important drawbacks. First, visual examination of skin colour is poor at quantifying subtle differences of erythema.[11] Namely, colour is a subjective and nonlinear sensory perception, because ocular sensitivity to visible light depends on wavelength and shows intraindividual variation.[24] Furthermore, skin colour is a mixture of "redness" from cutaneous blood flow, and "tanning" derived from epidermal melanin, imposing challenges in isolating these components by visual inspection only.[8] So, visual erythema scoring is a subjective

and quite unreliable method in therapeutic monitoring, and its robustness, also by experienced dermatologists, can therefore be questioned.[5]

Various methods to objectify erythema have already been tested in dermatological research.[25] These studies showed that quantified erythema values correlate well with clinical scores, both in rosacea,[11-15,17,18] and in other inflammatory skin diseases.[26,27] Despite their promising value, none of these proposed methods have so far been implemented in daily clinical practice. We hypothesize that this is because they are too complex, expensive, or time-consuming to use. In this study, we evaluated a very simple method for erythema monitoring in rosacea using ImageJ, which is easy to use, freely available, and widely accepted for image analysis.[28] Interobserver correlation is high, making our results reproducible; no extensive training is needed, so calculations can be easily performed by clinicians who are unexperienced with image software. Moreover, ImageJ allows temporal monitoring of exactly the same skin location due to ROI saving options. The ROI can be easily adjusted for analysis of both small as well as large skin areas to obtain distributions maps, only requiring sufficient image resolution.[8] This is a great advantage over spectrophotometric measurements which are point measurements, also prohibiting use in small skin locations (e.g., the nose).[22] In this study, we chose a circular ROI with clearly visible facial erythema, serving as a representative piece of lesional skin. Moreover, this is a non-contact method, so it does not change skin colour due to capillary construction and consecutive blanching of the skin.[8,9,22] In addition to avoiding pressure to the skin, there is no need to apply an instrument to the lesional skin, having hygienic disadvantages.[9]

In this study, both RGB and CIELAB colour space were used to calculate redness. RGB indicates how a colour of an object "appears", corresponding to the three types of colour sensors (cones) in the human eye.[9] Using RGB, no differences in R/G ratio were measured, corresponding to earlier work focussing on rosacea severity.[12] An explanation for this could be that RGB values are not only influenced by colour but also by brightness, which probably varied slightly between photographs. With CIELAB colour space, one does not encounter this problem, as brightness is separated from the a^*-axis of the colour space. CIELAB provides the perception of colour to a human observer, and closely approximates and linearly correlates with the response of the eye.[6,9,20,22] Despite the relatively low R_s, a clear relationship between lesional a^* values and clinical scores was seen. The weak correlation may be caused by the subjectivity of the determined clinical scores. As there is no noninvasive golden standard tool to provide the "real" erythema value, there was no other suitable noninvasive technique to compare all our results to.[15]

It is important to take into account that a^* represents erythema of both physiologic and pathologic cause,[12] as it correlates with haemoglobin, skin blood flow and vascularization.[8,15,22,29-31] However, the correlation of a^* with haemoglobin is almost

linear, and independent of the amount of melanin.[10] Furthermore, erythema values can be influenced by various individual -and environmental related variables such as age, medication, caffeine intake, orthostatic effects, physical activity, regional and seasonal variation, ambient temperature and humidity rate, and lighting inconsistencies.[9,10,20,30] In this study, photographs were taken under standardized conditions, four time points were included per patient, and a non-lesional site was measured as an internal control. We deliberately chose not to correct for other possible influencing variables, because this limits clinical application immensely; still, the quantified a^* and Δa^* values showed a clear correlating trend with clinical scores, and both parameters decreased significantly during treatment with ivermectin. This is probably caused by a reduction in inflammation, as topical ivermectin has moderate-to-high certainty evidence for reducing papules and pustules in rosacea.[21] However, even after 16 weeks of treatment, lesional erythema values remained higher than non-lesional values, suggesting that persisting erythema is a partly non-inflammatory feature (e.g. due to telangiectasias).

Our method appears to be rapid, and can in our opinion compete with clinical assessment, which is highly recommended for application in clinical practice. Moreover, retrospective analysis of images is possible, preventing the use of extra time in the consultation room. It could possibly be expanded to quantify erythema in a wide range of inflammatory dermatoses, such as rosacea, atopic dermatitis and psoriasis. We suggest to use only a^* values, and not Δa^*, because correlations of both parameters with clinical scores are comparable, but a^* determination is faster than Δa^*. We recommend applying standardized, consistent, photography conditions in a studio setting.

CONCLUSION

The tested image-based software tool is a simple, free, rapid, and reproducible method to objectify and monitor erythema in rosacea patients during treatment. The only two requirements necessary for erythema analysis are: (a) ImageJ software, able to convert RGB images to CIELAB colour space and to quantify colour intensity (a^*) of a selected ROI; (b) clinical photographs, taken under standardized conditions in a studio. The photographs allow retrospective analysis, evaluation of large and small lesions, and discrimination of subtle redness differences. We recommend using lesional a^* in follow-up of erythema in inflammatory diseases in daily clinical practice (Table 3). We believe that this method is easily applicable for clinicians, and in the future, ideally would replace determination of subjective clinical scoring.

Table 3. Step-by-step guideline for erythema quantification using ImageJ, recommended for daily clinical practice.

1. Drag original JPG photograph in ImageJ.
2. Split image into CIELAB colour space: **Image → Color → RGB to CIELAB.**
3. Scroll to second image (*a**).
   - *For first visit:*
   Choose a region of interest (ROI) including lesional skin. Outline ROI with **'Freehand selections'**. Make sure
   to avoid any skin colour inconsistency due to pen lines, hair, tattoos, jewellery etc. inside ROI.
   Analyse → Measure: record mean *a** of lesional skin.
   Save ROI: **Analyse → Tools → ROI manager.** Click Add **[t] → More → Save.**
   - *For follow-up visits:*
   Drag saved ROI into ImageJ; ROI is placed into the *a** image.
   Analyse → Measure: record mean *a** of lesional skin.
4. Transfer all recorded values to a digital database for data analysis.

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CHAPTER 3.4

Rosacea and use of continuous positive airway pressure mask for obstructive sleep apnea syndrome: report of five cases

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ABSTRACT

Rosacea is a chronic inflammatory skin disease of unknown etiology. We noticed a series of patients who were diagnosed with rosacea as well as obstructive sleep apnea syndrome (OSAS), for which they used a continuous positive airway pressure (CPAP) mask. This case series aims to give insight in the possible relationship between rosacea and the use of a CPAP mask for OSAS. We present five patients with OSAS who developed or worsened rosacea symptoms after use of a CPAP mask covering nose and mouth. Two patients showed centrofacial symptoms consistent with the shape of the CPAP mask; three patients had nasal cutaneous symptoms. It is postulated that the occlusive effect of the CPAP mask, increasing skin humidity and temperature, can induce primary symptoms in patients with an underlying sensibility for rosacea. This could have implications for choice of CPAP mask type and topical therapeutic options for rosacea.

INTRODUCTION

Rosacea is a common chronic inflammatory skin disease presenting with erythema, papules, pustules and telangiectasias of predominantly the cheeks, forehead, chin and nose.[1,2] Four rosacea subtypes are described; erythematotelangiectatic rosacea (ETR), papulopustular rosacea (PPR), phymatous rosacea, and ocular rosacea. The exact pathophysiology of rosacea remains unknown; many factors seem to play a role in disease development.[3] In addition, it is associated with various chronic systemic diseases, like gastroesophageal reflux disease, and hypertension.[4] We noticed multiple rosacea patients at our outpatient clinic with concurrent obstructive sleep apnea syndrome (OSAS) using a continuous positive airway pressure (CPAP) mask. In OSAS, the upper airways collapse repetitively during sleep, causing hypoxia, sleep disruption, and daily fatigue.[5] A CPAP mask serves as a pneumatic stabilizer in moderate to severe OSAS. Here, we report five patients with rosacea and OSAS with use of a CPAP mask. We propose mechanisms to explain a possible causality between these two entities.

CASE REPORT

Case 1

A 60-year-old female with OSAS, obesity (body mass index, 36.8 kg/m^2) and hypertension (for which she was prescribed a thiazide diuretic), experienced sunlight-aggravated periodical facial sensations of burning, tightness, itching, pain, erythema, pustules and periorbital edema since 2010. She also started full-face CPAP ventilation (covering nose and mouth) every night in 2010. Clinical examination in 2016 revealed perioral erythematous papules consistent with the shape of her CPAP mask, and excoriated papules on the cheeks and forehead. Histology showed chronical focal active perivascular and perifollicular infiltrates without signs of contact dermatitis; consistent with rosacea and/or folliculitis. Based on the clinical picture, the diagnosis PPR was made. Previous treatments included topical metronidazole, azelaic acid and ivermectin, and oral tetracyclines, all giving short-term improvement of skin symptoms. She stated that her facial symptoms started after initial use of the mask, with symptom deterioration after prolonged usage. Topical metronidazole application in the evening was difficult because it caused displacement of the CPAP mask and consequently air leakage. After discontinuing CPAP ventilation in 2017 due to the disappearance of apnea complaints caused by substantial weight reduction, symptoms of rosacea improved drastically. Topical ivermectin monotherapy was provided since then.

Case 2

A 61-year-old female with mild OSAS suffered from mild heat-aggravated ETR and PPR since the age of 30. This patient was referred in 2015 because of development of an elevated spot on the right nasal ala. Also in 2015, this patient starting using a full-

face CPAP mask every other night. Clinical examination revealed a mildly edematous right nasal ala, and mild erythema and telangiectasias on cheeks and forehead. No papules, pustules or signs of rhinophyma were present. The nasal edema was linked to active rosacea (morbus Morbihan) and expanded to both alae during follow-up in 2016. Treatment with topical metronidazole and azelaic acid and oral tetracyclines were non-efficacious; manual lymphatic drainage is currently being considered. Nowadays, this patient is still using the full-face CPAP mask. (Intra)nasal devices were tried but not successful (less effective and painful).

Case 3

A 74-year-old-male with mild-severe OSAS, hypertension and coronary heart disease (for which he was prescribed a statin, angiotensin-converting enzyme (ACE)-inhibitor, thiazide diuretic and a beta-blocker), started using a full-face CPAP mask in 2012. This patient suffered from recurrent nasal inflammation since multiple years with periodical nasal thickening, erythema, pain, and outflow of pus. Symptoms aggravated in 2017. Physical examination in 2018 revealed prominent nasal follicle openings, sebum gland hypertrophy and open comedones, consistent with rhinophyma, together with multiple telangiectasias on the cheeks and forehead. Oral doxycyclin was prescribed, followed by surgical dermabrasion of the nasal skin one month later. This regimen resulted in decrease of nasal pustules and erythema. Metronidazole maintenance therapy was provided one month after surgery. In the same period, CPAP therapy was discontinued due to air leakage and replaced by a mandibular reposition device. The patient could not clearly indicate whether the change of device influenced rhinophyma symptoms.

Case 4

A 59-year-old male with OSAS and hypercholesterolemia (for which no therapy was used) experienced a sunlight-aggravated fatty skin of the nose for approximately 20 years. He used a selective serotonin-reuptake inhibitor for depression. He started wearing a full-face CPAP mask every night in 2015. Since 2017, small bumps on the nose, roughening of nasal skin and periodical pustules were noticed. Previous topical therapies (corticosteroids, metronidazole) were ineffective. Clinical examination in 2018 revealed diffuse nasal fatty erythema, telangiectasias, pustules and fibrosis, and mild diffuse erythema and yellowish desquamation at the nasolabial folds and eyebrows. Patient's rhinophyma with concurrent seborrheic dermatitis were treated with topical ivermectin and ketoconazole and oral doxycyclin, resulting in improvement of skin symptoms.

Case 5

A 48-year-old male with OSAS, obesity, hypertension and diabetes mellitus type 2 (for which he used blood glucose lowering drugs, and a statin, ACE-inhibitor, thiazide diuretic, calcium antagonist, beta-blocker, and low-dose acetylic acid) started using

a full-face CPAP mask in 2015. In the same period, symptoms of recurrent pustules, nasal thickening, and dermal inflammation of the perioral region and nose appeared. Clinical examination in 2017 revealed erythema, papules and pustules between the eyebrows, on the nose and in the nasolabial fold; moreover, nasal fibrosis was present. Aforementioned skin symptoms were consistent with the shape of the CPAP mask (Fig. 1). The clinical diagnosis PPR with rhinophyma was made. Initial treatment with topical metronidazole was ineffective; oral tetracyclines reduced skin inflammation thereafter. As nasal fibrosis remained, surgical dermabrasion of nasal skin was performed. He suggested that his skin symptoms were caused by inadequate cleaning of his CPAP mask; nowadays, he cleans his mask more frequently. Moreover, he is actively trying to lose weight.

Figure 1. Patient with rhinophyma and papulopustular rosacea. Skin symptoms were consistent with the shape of the CPAP mask. Informed consent was obtained from the patient for publication of the photographic materials.

A summary of the cases is presented in Table 1.

Table 1. Summary of reported cases of rosacea and CPAP mask for OSAS.

Case	Age/sex	Rosacea type (since)	Mask type (since)	Comments
1	60/female	PPR (2010)	Full-face (2010)*	Stopped using CPAP in 2017, rosacea improved since then
2	61/female	ETR, morbus Morbihan (2016)	Full-face, nasal, intranasal (2015)	Mild ETR and PPR since 90's
3	74/male	Rhinophyma, mild (2018)	Full-face (2012)	Mild nasal symptoms for several years, stopped using CPAP in 2018
4	59/male	Rhinophyma (2017)	Full-face (2015)	Fatty nasal skin for 20 years
5	48/male	PPR, rhinophyma (2015)	Full-face (2015)	Skin symptoms consistent with shape of CPAP mask

* Covering nose and mouth. CPAP, continuous positive airway pressure. OSAS, obstructive sleep apnea syndrome. PPR, papulopustular rosacea. ETR, erythematoteleangiectatic rosacea

DISCUSSION

We presented five patients with development or worsening of rosacea after use of full-face CPAP masks covering nose and mouth for OSAS. Two patients were diagnosed with PPR, one with ETR with morbus Morbihan and three patients with rhinophyma. Three patients had nasal skin symptoms and two patients showed centrofacial symptoms consistent with the shape of the CPAP mask. Here, several hypotheses are proposed to explain the surprising similarities between our cases.

First, temperature may play a role; a significant higher rosacea frequency was found in females exposed to tandoor heat.[6] Possibly, full-face CPAP masks cause a warm facial environment, inducing heat-specific transient receptor potential (TRP) receptors on keratinocytes, resulting in inflammation and vasodilatation.[1,3] Also *Demodex* mites, part of normal facial skin flora, may be involved in this process, as they seem to play a role in the development of rosacea.[3] Previous work showed increased immune-stimulatory protein production by *Demodex*-containing bacteria at higher temperatures.[7]

Secondly, occlusion could be a factor. A CPAP device is equipped with a humidifier pumping water into the mask to reduce dry mouth and nasal congestion. This water may cause a humid, occlusive layer onto facial skin. This effect can be enhanced by application of topical therapies or bad mask hygiene, resulting in sebum gland obstruction and inducing primary symptoms of an underlying sensibility for rosacea. We therefore advice to apply topical therapies as far as possible before putting on the mask. Furthermore, we recommend patients to follow the general cleaning guidelines for the CPAP apparatus.

Lastly, comorbidities may play a role. OSAS is associated with metabolic syndrome, and diabetes and hypertension are frequent comorbitities.[8,9] In OSAS patients with those comorbidities, changed cortisole levels and inflammatory mediators have been found that could declare an altered sensitivity for general inflammation.[10,11] Interestingly, rosacea is also associated with cardiovascular risk factors and metabolic syndrome.[4,12,13] In our cases, four of five patients had cardiovascular and/or metabolic comorbidities. Possibly, those comorbidities are an important link between CPAP use for OSAS and rosacea.

It is important to note that other co-factors could influence rosacea symptoms. Due to the retrospective character and limited amount of subjects of this report, we did not study those factors in more detail. First, various food components like pepper, alcohol and hot beverages can aggravate rosacea by upregulating TRP channels.[1,3,14] Dietary factors did not seem to be of influence in our patients, as skin symptoms diminished after removal of the CPAP mask. Second, photosensitizing medication, e.g. chlorthalidone and metoprolol, could increase rosacea severity. Lastly, contact allergies coexist in rosacea,[15] but no clinical/histological signs of contact dermatitis were present in our patients.

In conclusion, we reported five patients with development or worsening of rosacea symptoms after use of a full-face CPAP mask covering nose and mouth for OSAS. We propose an occlusive effect of the CPAP mask on the skin, increasing skin humidity and temperature, which induces primary symptoms of an underlying sensibility for rosacea. In addition, OSAS and its cardiovascular/metabolic comorbidities could play a role in the development of rosacea symptoms. In future, more attention should be paid to patients who develop rosacea when using CPAP masks. This could have implications for choice of CPAP mask type and topical therapeutic treatment for rosacea.

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CHAPTER 3.5

Use of beta-blockers for rosacea associated facial erythema and flushing: a systematic review and update on proposed mode of action

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ABSTRACT

Background

Flushing and erythema are frequent skin symptoms in rosacea. Because their adequate treatment remains a clinical challenge, new treatment options are explored, such as oral β-blockers.

Objective

To evaluate the efficacy of oral β-blockers for rosacea-associated facial flushing and erythema.

Methods

PubMed, Embase, Web of Science, and Cochrane Library were systematically searched, including studies providing original data on the efficacy of oral β-blockers in rosacea patients with facial flushing and/or persistent erythema. Risk of bias was assessed using the Cochrane Risk of Bias tool, Newcastle-Ottawa scale, and Quality in Prognosis Studies tool.

Results

Nine studies evaluating the use of carvedilol, propranolol, nadolol, and β-blockers in general were included. Articles studying carvedilol and propranolol showed a large reduction of erythema and flushing during treatment with a rapid onset of symptom control. Bradycardia and hypotension were the most commonly described adverse events.

Limitations

Most studies had a retrospective design with a small sample size, and outcome measurement was often subjective.

Conclusion

Oral β-blockers could be an effective treatment option for rosacea patients with facial erythema and flushing that does not respond to conventional therapy. Larger prospective trials with objective outcome assessment are needed to validate the promising results of these studies.

INTRODUCTION

Flushing and persistent erythema are common rosacea symptoms.[1,2] In contrast to effective treatment options targeting inflammation in rosacea, diminishing erythema and flushing remains a clinical challenge.[3,4] The etiology of increased blood flow in rosacea is complex and probably multifactorial; both vessel dilation and neuronal, inflammatory, and hormonal pathways, which can be enhanced by various external triggers, seem to be involved.[5-7] The only approved treatments for facial erythema in rosacea are topical brimonidine and oxymetazoline, two selective α-adrenergic receptor agonists.[8-10] Although effective in some cases, poor response and rebound erythema are common, especially for brimonidine.[10-15] Their vasomotor target is, however, interesting, resulting in local vasoconstriction. Because skin appearance has a significant impact on quality of life, the importance of new approaches for facial erythema and flushing has become clear.[16-18]

A possible therapeutic option not yet approved for persistent erythema and flushing is treatment with oral *β*-blockers, which antagonize the effects of sympathetic nerve stimulation and circulating endogenous catecholamines at adrenoreceptors.[19,20] Three types of adrenoreceptors exist; β_1-receptors are mainly located in the heart,[21] β_2-receptors in the lungs, gastrointestinal tract, blood vessels, and skin (keratinocytes, fibroblasts),[22-25] and α_1-receptors are, among other locations, found in the smooth muscles of cutaneous blood vessels. In rosacea, *β*-blockers are believed to reduce erythema by blocking β_2-adrenergic receptors on smooth muscles of cutaneous arterial blood vessels, causing vasoconstriction.[26] Moreover, they may reduce anxiety and tachycardia, which can exacerbate flushing reactions.[27-30]

The aim of this systematic review was to elucidate the efficacy of oral *β*-blockers for flushing and persistent facial erythema in rosacea and to provide recommendations for clinical practice.

MATERIALS AND METHODS

The study protocol was registered in PROSPERO (identification 159025).[31] A systematic literature search following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines[32] was conducted in PubMed, Embase, Cochrane Library, and Web of Science. Search terms were *rosacea, flushing, facial erythema,* and *beta-blockers*, along with all possible synonyms. Oral *β*-blocker types were extracted from a recent Cochrane review[33] and by exploring their Medical Subject Heading terms. Search strategy details can be found in Table S1 (Supplementary Materials). We included studies conducted in adults with cutaneous facial rosacea that provided original data on use of oral *β*-blockers for rosacea-associated flushing and/or erythema

(Table S2, Supplementary Materials). Physical modalities such as laser therapy also act on the vascular component but were beyond the scope of this article.[3,34]

All databases were searched to include published studies from date of inception until November 20, 2019. Titles and abstracts were screened for relevance by two independent reviewers (JGML and JIO). Next, full texts were critically assessed for eligibility by the same reviewers. Missing full texts were requested via the Radboud University Medical Library. In both phases, differences between the reviewers regarding inclusion were resolved by discussion. Excluded were articles involving patients younger than 16 years; ocular, extrafacial, or drug-induced rosacea; drug-induced flushing; in vitro and animal studies; studies in languages other than English, German or Dutch; meta-analyses, (systematic) reviews, and abstracts of congresses, or those with unavailable full texts. The reference lists of included articles were checked for relevant articles not identified by the initial search.

Extracted study characteristics included study design; number of participants; rosacea symptoms; β-blocker type, dose, and treatment duration; erythema/flushing assessment method; study findings; and adverse events. A narrative synthesis was conducted for each β-blocker separately. Risk of bias was assessed by two reviewers (JGML and JIO), with disagreements resolved by discussion. The Cochrane Risk of Bias tool was used for assessment of risk of bias in randomized controlled trials (RCTs), with studies graded as having low, high, or unclear risk of bias.[35] For case-control studies, the Newcastle-Ottawa Scale was used.[36] For cohort studies without a control group (including case reports and case series), the Quality in Prognosis Studies tool was used.[37] For the Quality in Prognosis Studies, the overall risk of bias for each of the studies was judged as (1) low, if there were a low risk of bias in all key domains; (2) unclear, if there was an unclear risk of bias for one or more key domains; and (3) high, if there was a high risk of bias for one or more key domains.

RESULTS

In total, 1941 articles were identified (Fig. 1). After duplicate removal, 1544 articles were screened, resulting in inclusion of 25 abstracts eligible for full-text screening. Finally, nine articles were included in this systematic review. Investigated β-blockers were carvedilol (n = 4),[26,38-40] propranolol (n = 3),[29,41,42] nadolol (n = 1),[30] and β-blockers in general (n = 1).[43] Among the included articles were 1 RCT, 1 cohort study, 1 case-control study, 3 case reports, and 3 case series. In the following sections and in Tables 1 and 2, the β-blockers included in this review are presented separately.

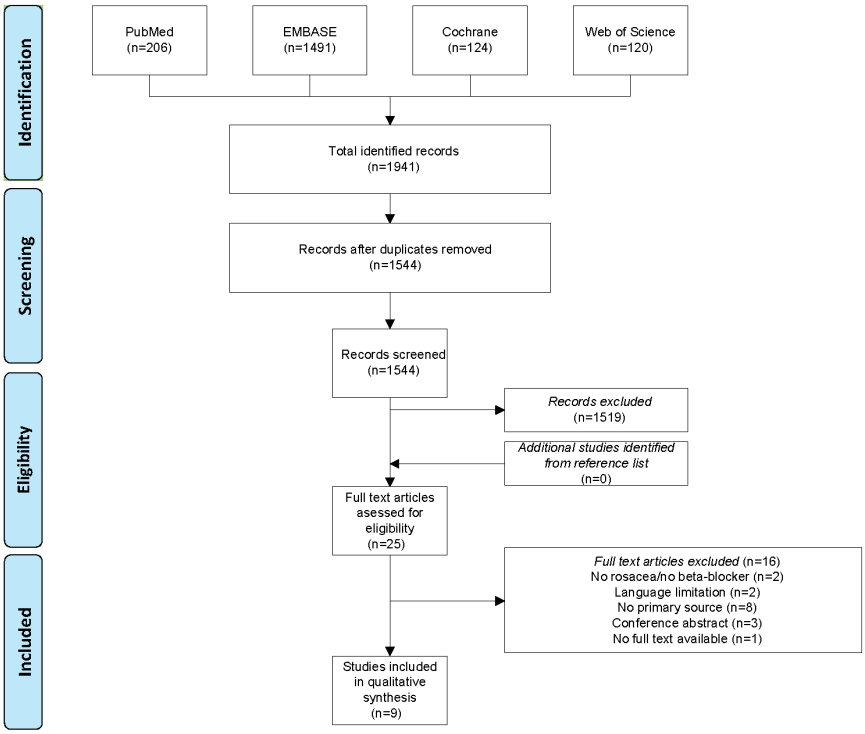

Figure 1. Flow chart: article selection process.

Nadolol

Nadolol is a nonselective β-blocker, blocking both β_1 and β_2-adrenergic receptors. Its use was described in 1 RCT.[30]

Fifteen patients with rosacea with erythema and flushing received nadolol 40 mg once daily or twice daily, or placebo, for 53 days. During this period, flushing challenges using warm water, ethanol, and nicotinic acid were performed. The intensity of flushing reactions was measured as degree of skin perfusion using laser Doppler velocimetry. No statistically significant differences in skin perfusion index were seen between nadolol and placebo. A modest to significant subjective improvement in number of occurrences, duration, and intensity of flushing with nadolol was found in 60% of patients; however, slight to definite worsening of flushing with nadolol was seen in 13% of patients as well.

Carvedilol

Carvedilol is a nonselective β-blocker with additional weak α_1-blocking activity. Four publications describing use of carvedilol in rosacea were identified.[26,38-40]

In a retrospective case study, five patients with moderate/severe rosacea-associated flushing or persistent erythema were treated with carvedilol titrated up to 12.5 mg twice daily for 6 months or longer.[39] All patients observed a reduction in facial erythema after 2 to 7 days from the start of treatment, and clinical erythema scores decreased in all patients at 6 months of treatment or longer. Erythema and facial flushing were still provoked by known triggers but to a much lower degree.

In another case series, carvedilol (3.125-6.25 mg, 2 or 3 times daily) was added to the regular medication (doxycycline, oral antihistamines/corticosteroids) of 11 patients with persistent erythema and facial flushing, and the dose was gradually titrated up to 31.25 mg/day.[26] This resulted in significant clinical erythema improvement within three weeks (range, 3-21 days) from the start of carvedilol, together with reduced cheek temperature and a large reduction in patient-assessed symptoms. Moreover, carvedilol allowed concurrent medications to be decreased in dosage or stopped.

Additionally, carvedilol usage was described in two case reports.[38,40] Clinical and patient-assessed improvement in erythema and flushing were seen within two weeks carvedilol treatment of 6.25 mg 2 or 3 times daily, with increased improvement thereafter using maintenance therapy of 6.25 mg 1 to 3 times daily for 23 months. Moreover, only 6.25 mg daily was needed in the summer.[38] Lee et al[40] showed clinical reduction of erythema and flushing after the start of carvedilol (6.25-12.5 mg thrice daily) together with brimonidine gel. Dermatoscopy showed polygonal vessel disappearance and blood vessel vasoconstriction after several months. Carvedilol was only needed intermittently afterwards during summer.

Propranolol

Propranolol is a traditional nonselective β-blocker; three studies focused on its use in rosacea.[29,41,42]

In a retrospective cohort study, nine patients with facial erythema and flushing received propranolol 10 mg 3 times daily, with doses increased as tolerated until symptoms improved, which appeared to be 20 to 40 mg 2 or 3 times daily.[29] Eight patients reported diminished symptoms and fewer flushing episodes while taking propranolol (duration of onset not described); one patient did not experience improvement but received only 100 mg thrice daily during one month without side effects and elected to discontinue propranolol thereafter.

Park et al[42] studied treatment with propranolol 10 mg thrice daily during 12 weeks in 22 patients with papulopustular and erythematotelangiectatic rosacea, and compared this with doxycycline (n = 15) and doxycycline and propranolol combination therapy (n = 26). The propranolol group showed a significant faster and larger reduction in clinical flushing scores compared to the other groups.

Finally, erythema and flushing improvement was observed in one patient already after one week of treatment with propranolol 40 mg once daily combined with minocycline and tranexamic acid.[41]

β-blockers in general

One study evaluated the association of β-blockers and the risk of developing rosacea by performing a case-control study with 53.927 patients with rosacea and 53.927 control individuals.[43] The paper does not describe which β-blocker types were included. A marginal decreased risk (odds ratio [OR] 0.91; 95% confidence interval [CI], 0.86-0.95) for current and long-term users of all β-blockers (OR, 0.89; 95% CI, 0.82-0.96) was found. Sensitivity analysis of the three most prescribed β-blockers in the United Kingdom (propranolol, atenolol, and bisoprolol) showed that the risk was slightly decreased for current users of atenolol (OR, 0.83; 95% CI, 0.74-0.94), and for current long-term users of bisoprolol (OR, 0.76; 95% CI, 0.60-0.96). Unexpectedly, no decreased risk for developing rosacea among propranolol users was found.

Adverse events

Seven included studies reported about adverse event occurrence (Table 2).[26,29,30,38,39,41,42] For nadolol, decreased heart rate and blood pressure was seen in 100% and 93% of patients, respectively.[30] For carvedilol, treatment was discontinued in 9.1% of patients (1 in 11) due to hypotension,[26] and dosage was adjusted in 20% of patients (1 in 5) because of vertigo and nausea.[39] Additionally, feeling of weakness (1 in 5)[39] and decreased blood pressure (1 in 11)[38] were noticed during carvedilol treatment. For propranolol, treatment was discontinued in 22% of patients (2 in 9) because of dizziness, bradycardia, and balance loss sensation.[29] Other reported, acceptable, side effects were decreased migraine headache severity (2 in 9), weight gain (1 in 9), fatigue (1 in 9), dyspepsia (1 in 22), and headache (1 in 22).[29,42] The case report from Kwon et al[41] reported no adverse events during treatment with propranolol.

Risk of bias

The number of patients of most studies was small, including multiple case series/case reports. Although the RCT was double-blinded, no information about the allocation sequence and blinding procedure was given (Fig. S1, Supplementary Materials). In the case-control study, results could be biased by the copresence of papules and pustules and not solely erythema and flushing. For cohort studies, which were mostly retrospective, the domains *outcome measurement* and *study confounding* carried the highest risk of bias (Fig. S2, Supplementary Materials). It was often not stated how and by whom the outcome measurements were determined. Moreover, potential confounders such as co-medication, rosacea type, cutaneous comorbidity, and rosacea-aggravating triggers were often insufficiently described or not taken into account.

Table 1. Summary of included studies evaluating the efficacy of *β*-blockers in rosacea patients with flushing and persistent facial erythema.

Authors	Study design	Participants, *n*	Rosacea symptoms	Treatment (type, dose, duration)	Erythema/flushing assessment	Study findings	AEs
Wilkin[30]	RCT	15 (F; 11, M: 4; age range 41-60 y)	ETR with flushing, erythema, telangiectasia	Study periods: A=18 days, B=17 days, C=18 days). Four groups: 1 (*n*=4): A+B=placebo, C=nadolol 40 mg QD; 2 (*n*=3): A+B=placebo, C=nadolol 40 mg BID; 3 (*n*=4): A=nadolol 40 mg QD, B+C=placebo; 4 (*n*=4): A=nadolol 40 mg BID, B+C=placebo. Flushing challenges: water (60 °C), ethanol, nicotinic acid at day 14+18 of period A+C.	RR, heart rate, laser Doppler velocimetry at right malar area for skin perfusion, patient's perception (flushing number, duration, intensity)	No statistically significant differences in perfusion index values between nadolol and placebo during flushing challenges. Modest to significant subjective improvement on spontaneous flushing with nadolol in 9 of 15 patients; slight to definite worsening of spontaneous flushing with nadolol in 2 of 15 patients.	Lower heart rate with nadolol (61±2.5/min) than placebo (70±2.5/min) in all patients. Lower mean arterial pressure with nadolol (76±2.5mmHg) than placebo (80±2.5mmHg) in 14 of 15 patients.
Pietschke and Schaller[39]	Retrospective case study	5 (F: 3, M: 2; age range 26-59 y)	Severe frequent flushing or persistent erythema	Carvedilol titrated up to 12.5 mg BID ≥ 6 months	Clinicians erythema assessment (CEA), patient's assessment (patients self-assessment (PSA), level of satisfaction, level of embarrassment)	All patients observed reduced facial erythema after 2-7 days of treatment. Mean CEA decreased from 3.4 at baseline to 0.4 after ≥6 months of treatment. Mean PSA decreased from 3.8 at baseline to 0.8 after ≥6 months of treatment. All 5 patients satisfied or highly satisfied with impact of carvedilol, with decreased level of embarrassment (3.4 to 0 after ≥6 months).	Vertigo and nausea (*n* = 1), feeling of weakness (*n* = 1)
Hsu and Lee[26]	Case series	11 (F:9, M; 2; age range 17-47 y)	Facial erythema	Carvedilol 3.125-6.25 mg BID or TID, titrated up to 31.25 mg/day, for 1 wk-28 months	Clinical photographs, cheek temperature, patients' assessment (VAS)	Significant clinical improvement within 3 weeks (range 3-21 days, mean 10.5 days), mean reduction of cheek temperature with 2.2°C, mean reduction of 6.3 on VAS scale	Hypotension (*n* = 1)

Table 1. Continued.

Authors	Study design	Participants, *n*	Rosacea symptoms	Treatment (type, dose, duration)	Erythema/flushing assessment	Study findings	AEs
Hsu and Lee[38]	Case report	1 (F, 48 y)	Flushing, persistent erythema, telangiectasia	Carvedilol (6.25 mg BID) for 1 week, then 6.25 mg QD, BID or TID for 23 months	Clinical (not further specified), cheek temperature, patients' assessment (VAS), RR	Dramatic improvement in erythema and telangiectasia within 2 weeks of treatment. Continuation of improvement with minimal erythema and only transient flushing episodes thereafter. Reduction in cheek temperature from 36.9°C to 30.0°C. Mean VAS reduction from 10 to 1.	Reduction in RR from 130/70 to 110/60 mmHg. No bradycardia.
Lee and Lee[40]	Case report	1 (F, 59 y)	ETR with transient and persistent erythema, telangiectasia	Carvedilol (6.25-12.5 mg TID; duration ND) and topical 0.33% brimonidine daily	Clinical (not further specified), dermoscopy	*Clinical:* persistent erythema resolved in 3 weeks after starting brimonidine. Only minimal telangiectasia at 6-month follow-up. Only mild flares over the 11 months. *Dermoscopy:* disappearance of polygonal vessels and significant vasoconstriction of larger blood vessels after months.	ND
Kwon et al[41]	Case report	1 (F, 37 y)	Flushing, persistent erythema, and marked telangiectasia	Propranolol (40 mg QD), minocycline (50 mg QD), and tranexamic acid (250 mg QD) for 1 month	Clinical (not further specified)	Noticeable improvement of erythema and subjective symptoms already after 1 week of treatment, persisting for 2 months.	No AE

Table 1. Continued.

Authors	Study design	Participants, *n*	Rosacea symptoms	Treatment (type, dose, duration)	Erythema/flushing assessment	Study findings	AEs
Park et al[42]	Prospective cohort study	63 (F: 47, M: 16; age range 16-76 y)	ETR or PPR with flushing	Propranolol 10 mg 3 TID (*n*=22), doxycycline 100 mg BID (*n*=15), propranolol 10 mg BID+doxycycline 100 mg BID (*n*=26). Duration: 12 weeks.	Investigator Global Assessment (IGA), rosacea clinical score (ARCS), Patient Global Assessment (PGA)	Decrease of IGA, ARCS and PGA in all three groups with no statistically significant differences. Propranolol group: flushing scores showed the biggest and fastest decrease after 12-week treatment compared to the other groups (statistically significant).	Propranolol-related: dyspepsia and headache (*n* = 1)
Craige and Cohen[29]	Retrospective cohort study	9 (F; 8, M: 1; age range 31-69 y)	Facial erythema, flushing	Propranolol (10 mg TID) with doses increased as tolerated until symptoms improved	Patient's perception (flushing episodes, symptoms, quality of life)	8 of 9 patients: diminished symptoms and flushing episodes. None had sufficient relief from 10 mg TID. Dose needed to control flushing: 20-40 mg BID or TID. 1 patient: no improved flushing, only received 10 mg TID for one month, elected to discontinue thereafter.	Bradycardia, fatigue, dizziness (*n* = 1), dizziness and sensation of balance loss (*n* = 1), mild weight gain (*n* = 1), decreased migraine headache severity (*n* = 2)
Spoendlin et al[43]	Case-control study	53.927 cases, 53.927 controls	Rosacea (PPR and ETR)	β-blockers in general	ND	Slightly decreased OR in current (OR=0.91) and long-term β-blocker users (OR=0.89). Slightly decreased OR during current use of atenolol across all strata of exposure duration (OR 0.74-0.83) and long-term current use of bisoprolol (OR 0.76). No decreased OR for propranolol use.	ND

AEs, adverse events. BID, twice daily. ETR, erythematotelangiectatic rosacea. F, female. M, male. OR, odds ratio. ND, not described. PPR, papulopustular rosacea. QD, once daily. RR, blood pressure. TID, thrice daily

Table 2. Reported adverse events in rosacea patients treated with oral β-blockers for flushing and persistent facial erythema.

Treatment	Reported adverse events
Nadolol	Decreased heart rate (*n* = 15)[30]; decreased blood pressure (*n* = 14)[30]
Carvedilol	Hypotension (*n* = 1)[26]; decreased blood pressure (*n* = 1)[38]; vertigo and nausea (*n* = 1)[39]; feeling of weakness (*n* = 1)[39]
Propranolol	Dizziness (*n* = 2)[29]; decreased migraine headache severity (*n* = 2)[29]; dyspepsia (n = 1)[42]; headache (*n* = 1)[42]; bradycardia (*n* = 1)[29]; sensation of balance loss (*n* = 1)[29]; weight gain (n = 1)[29]; fatigue (*n* = 1)[29]

CONCLUSIONS

Diminishing erythema and flushing in rosacea is challenging, because it hardly responds to conventional anti-inflammatory treatment. Patients in the included studies often had an extensive history of ineffective topical, oral, and/or physical treatments. Most studies showed improved erythema and flushing after initiation of oral β-blockers. The evidence was highest for carvedilol and propranolol, two nonselective β-blockers. Unfortunately, only a small selection of available β-blocker types was examined.

The most common adverse effects of nonselective β-blockers are bradycardia, hypotension, bronchospasm, dizziness, somnolence, and fatigue.[20,44] One should be aware that β-blockers may exacerbate asthma and psoriasis.[45-47] Contraindications to β-blockers are congestive heart failure, cardiogenic shock, sinus bradycardia of less than 50 beats/minute, atrioventricular block, hyperactive airway disease, and Raynaud disease.[19] It is important to monitor patients for adverse events, especially blood pressure and heart rate.[38]

Compared to other nonselective β-blockers, carvedilol and propranolol possibly have additional antioxidant and anti-inflammatory actions.[26,40,48,49] This may be beneficial in rosacea treatment, because reactive oxygen species released by inflammatory cells may play a role in disease development.[50-52] Carvedilol is usually well tolerated, even in elderly patients with heart failure.[53] Additionally, it results in fewer adverse effects, such as hypotension and bradycardia, than traditional β-blockers, which may be a limiting factor in normotensive patients.[38,54] Propranolol can cause additional diarrhoea, nausea, and sexual dysfunction in males,[55] and it is recommended that it be started at a lower dosage in geriatric patients and those with renal or hepatic disease.[20] Nadolol offers the advantage of a once-daily dosage because of its long plasma half-life (14-24 hours).[30] β-blockers dosages for reducing facial erythema are generally lower compared to the maintenance dose needed in hypertension (nadolol: 40-80 mg vs 80-320 mg daily;[30] carvedilol: 6.25-37.5 mg vs 25 mg daily;[26,38-40] propranolol: 30-120 mg vs 160-320 mg daily[29,41,42]). The efficacy of topical β-blockers such as timolol, being effective in various vascular dermatoses,[20] has not yet been investigated in rosacea.

Several studies have investigated other systemic medications antagonizing erythema and flushing in rosacea. Clonidine, an α_2-adrenergic agonist, did not suppress erythema and flushing.[56,57] Also, rilmenidine, a central hypotensive drug, did not improve facial flushing compared to placebo.[58] Ondansetron, a serotonin antagonist, improved persistent erythema and flushing in two patients.[59] Naloxone, an opioid receptor antagonist, reduced alcohol-induced flushing, but has many side effects.[60] Otherwise, phentolamine, an α-adrenergic antagonist, even increased blood flow during exercise in frequent blushers.[28] The aforementioned medications, therefore, seem largely dissatisfying until today.

The quality of included studies was relatively low, and interstudy outcome variability was large. It was not possible to perform a meta-analysis, because erythema and flushing were assessed using a wide spectrum of mostly subjective clinical and patient-based scores, and method standardization was often missing. The evaluation of facial erythema by visual assessment alone lacks objectivity and precision, and it is prone to inter- and intra-observer variability.[61-63] This makes comparison of individual study outcomes challenging. Simple, standardized, and objective erythema and flushing assessment, such as spectrophotometry and computer-aided image analysis, are advisable.[64]

To conclude, oral nonselective *β*-blockers could be an effective treatment option for rosacea patients with persistent facial erythema and flushing. Currently, most evidence is available for carvedilol and propranolol. Large, prospective, clinical trials are warranted to validate the data of these small studies. Researchers should further focus on the determination of the optimal dosage, treatment duration, and long-term therapeutic effects for adequate treatment of erythema and flushing in rosacea.

ACKNOWLEDGEMENTS

We would like to thank A.H.J. Tillema for her contribution in developing the search strategy.

SUPPLEMENTARY MATERIALS

Table S1. Search strategy.

Pubmed No limitations	**EMBASE** No limitations
("Rosacea"[Mesh] OR rosacea*[tiab] OR "Flushing"[Mesh] OR flush*[tiab] OR ((face[tiab] OR facial[tiab]) AND (erythem*[tiab] OR redness[tiab]))) AND ("Adrenergic beta-Antagonists"[Mesh] OR "Adrenergic beta-Antagonists" [Pharmacological Action] OR Acebutolol[tiab] OR adaprolol[tiab] OR adimolol[tiab] OR afurolol[tiab] OR Alprenolol[tiab] OR amosulalol[tiab] OR arotinolol[tiab] OR Atenolol[tiab] OR befunolol[tiab] OR bornaprolol[tiab] OR brefonalol[tiab] OR bucumolol[tiab] OR bunitrolol[tiab] OR Betaxolol[tiab] OR bevantolol[tiab] OR Bisoprolol[tiab] OR bopindolol[tiab] OR bromoacetylalprenololmenthane[tiab] OR bucindolol[tiab] OR bufetolol[tiab] OR bufuralol[tiab] OR Bunolol[tiab] OR Bupranolol[tiab] OR butofilolol[tiab] OR Butoxamine[tiab] OR chlortalidone cloranolol[tiab] OR cyanoiodopindolol[tiab] OR carazolol[tiab] OR carpindolol OR Carteolol[tiab] OR Carvedilol[tiab] OR Celiprolol[tiab] OR cyanopindolol[tiab] OR deacetylmetipranolol[tiab] OR dexpropranolol OR diacetolol[tiab] OR dichlorisoprenaline OR dilevalol[tiab] OR Dihydroalprenolol[tiab] OR diprafenone OR epanolol[tiab] OR ersentilide OR esmolol[tiab] OR exaprolol[tiab] OR falintolol[tiab] OR flusoxolol[tiab] OR flestolol[tiab] OR hydroxybenzylpinodolol[tiab] OR hydroxycarteolol[tiab] OR hydroxymetoprolol[tiab] OR iodocyanopindolol[tiab] OR iodopindolol[tiab] OR iprocrolol[tiab] OR isamoltane OR isoxaprolol[tiab] OR indenolol[tiab] OR levomoprolol[tiab] OR Iodocyanopindolol[tiab] OR Labetalol[tiab] OR landiolol[tiab] OR Levobunolol[tiab] OR medroxalol[tiab] OR mepindolol[tiab] OR methylthiopropranolol[tiab] OR moprolol[tiab] OR mercuderamide OR Metipranolol[tiab] OR Metoprolol[tiab] OR Nadolol[tiab] OR nevibolol[tiab] OR nipradilol[tiab] OR nifenalol[tiab] OR oberadilol OR Oxprenolol[tiab] OR pafenolol[tiab] OR pamatolol[tiab] OR primidolol[tiab] OR procinolol[tiab] OR pronetalol[tiab] OR proxodolol[tiab] OR Penbutolol[tiab] OR Pindolol[tiab] OR Practolol[tiab] OR prizidilol[tiab] OR Propranolol[tiab] OR ridazolol[tiab] OR salcardolol[tiab]OR Sotalol[tiab] OR soquinolol[tiab] OR spirendolol[tiab] OR tazolol OR talinolol[tiab] OR tienoxolol[tiab] OR tolamolol[tiab] OR toliprolol[tiab] OR tribendilol[tiab] OR tertatolol[tiab] OR tilisolol[tiab] OR Timolol[tiab] OR trasitensin OR tobanum[tiab] OR xibenolol[tiab] OR zoleprodolol OR beta-Antagonist*[tiab] OR beta-Adrenoceptor Antagonist*[tiab] OR beta-Blocker*[tiab] OR beta-Adrenergic Receptor Blockader*[tiab] OR beta-Adrenergic Blocking Agent*[tiab] OR beta Adrenergic Blocker*[tiab] OR beta-Adrenergic Antagonist*[tiab] OR Adrenergic beta-Receptor Blockader*[tiab])	(rosacea/ OR flushing/ OR face erythema/ or (rosacea* OR flush* OR ((face OR facial) AND (erythem* OR redness))).ti,ab,kw.) AND (beta adrenergic receptor blocking agent/ OR Acebutolol.ti,ab,kw. OR adaprolol.ti,ab,kw. OR adimolol.ti,ab,kw. OR afurolol.ti,ab,kw. OR Alprenolol.ti,ab,kw. OR amosulalol.ti,ab,kw. OR arotinolol.ti,ab,kw. OR Atenolol.ti,ab,kw. OR befunolol.ti,ab,kw. OR bornaprolol.ti,ab,kw. OR brefonalol.ti,ab,kw. OR bucumolol.ti,ab,kw. OR bunitrolol.ti,ab,kw. OR Betaxolol.ti,ab,kw. OR bevantolol.ti,ab,kw. OR Bisoprolol.ti,ab,kw. OR bopindolol.ti,ab,kw. OR bromoacetylalprenololmenthane.ti,ab,kw. OR bucindolol.ti,ab,kw. OR bufetolol.ti,ab,kw. OR bufuralol.ti,ab,kw. OR Bunolol.ti,ab,kw. OR Bupranolol.ti,ab,kw. OR butofilolol.ti,ab,kw. OR Butoxamine.ti,ab,kw. OR chlortalidone cloranolol.ti,ab,kw. OR cyanoiodopindolol.ti,ab,kw. OR carazolol.ti,ab,kw. OR carpindolol OR Carteolol.ti,ab,kw. OR Carvedilol.ti,ab,kw. OR Celiprolol.ti,ab,kw. OR cyanopindolol.ti,ab,kw. OR deacetylmetipranolol.ti,ab,kw. OR dexpropranolol OR diacetolol.ti,ab,kw. OR dichlorisoprenaline OR dilevalol.ti,ab,kw. OR Dihydroalprenolol.ti,ab,kw. OR diprafenone OR epanolol.ti,ab,kw. OR ersentilide OR esmolol.ti,ab,kw. OR exaprolol.ti,ab,kw. OR falintolol.ti,ab,kw. OR flusoxolol.ti,ab,kw. OR flestolol.ti,ab,kw. OR hydroxybenzylpinodolol.ti,ab,kw. OR hydroxycarteolol.ti,ab,kw. OR hydroxymetoprolol.ti,ab,kw. OR iodocyanopindolol.ti,ab,kw. OR iodopindolol.ti,ab,kw. OR iprocrolol.ti,ab,kw. OR isamoltane OR isoxaprolol.ti,ab,kw. OR indenolol.ti,ab,kw. OR levomoprolol.ti,ab,kw. OR Iodocyanopindolol.ti,ab,kw. OR Labetalol.ti,ab,kw. OR landiolol.ti,ab,kw. OR Levobunolol.ti,ab,kw. OR medroxalol.ti,ab,kw. OR mepindolol.ti,ab,kw. OR methylthiopropranolol.ti,ab,kw. OR moprolol.ti,ab,kw. OR mercuderamide OR Metipranolol.ti,ab,kw. OR Metoprolol.ti,ab,kw. OR Nadolol.ti,ab,kw. OR nevibolol.ti,ab,kw. OR nipradilol.ti,ab,kw. OR nifenalol.ti,ab,kw. OR oberadilol OR Oxprenolol.ti,ab,kw. OR pafenolol.ti,ab,kw. OR pamatolol.ti,ab,kw. OR primidolol.ti,ab,kw. OR procinolol.ti,ab,kw. OR pronetalol.ti,ab,kw. OR proxodolol.ti,ab,kw. OR Penbutolol.ti,ab,kw. OR Pindolol.ti,ab,kw. OR Practolol.ti,ab,kw. OR prizidilol.ti,ab,kw. OR Propranolol.ti,ab,kw. OR ridazolol.ti,ab,kw. OR salcardolol.ti,ab,kw.OR Sotalol.ti,ab,kw. OR soquinolol.ti,ab,kw. OR spirendolol.ti,ab,kw. OR tazolol OR talinolol.ti,ab,kw. OR tienoxolol.ti,ab,kw. OR tolamolol.ti,ab,kw. OR toliprolol.ti,ab,kw. OR tribendilol.ti,ab,kw. OR tertatolol.ti,ab,kw. OR tilisolol.ti,ab,kw. OR Timolol.ti,ab,kw. OR trasitensin OR tobanum.ti,ab,kw. OR xibenolol.ti,ab,kw. OR zoleprodolol OR beta-Antagonist*.ti,ab,kw. OR beta-Adrenoceptor Antagonist*.ti,ab,kw. OR beta-Blocker*.ti,ab,kw. OR beta-Adrenergic Receptor Blockader*.ti,ab,kw. OR beta-Adrenergic Blocking Agent*.ti,ab,kw. OR beta Adrenergic Blocker*.ti,ab,kw. OR beta-Adrenergic Antagonist*.ti,ab,kw. OR Adrenergic beta-Receptor Blockader*.ti,ab,kw.)
Until 20-11-2019: **206 hits**	Until 20-11-2019: **1491 hits**

Table S1. Continued.

Cochrane Library No limitations	**Web of Science** No limitations
1. MeSH Rosacea 2. MeSH Flushing 3. (rosacea* OR flush* OR ((face OR facial) AND (erythem* OR redness))):ti,ab,kw 4. MeSH Adrenergic beta-Antagonists 5. (Acebutolol OR adaprolol OR adimolol OR afurolol OR Alprenolol OR amosulalol OR arotinolol OR Atenolol OR befunolol OR bornaprolol OR brefonalol OR bucumolol OR bunitrolol OR Betaxolol OR bevantolol OR Bisoprolol OR bopindolol OR bromoacetylalprenololmenthane OR bucindolol OR bufetolol OR bufuralol OR Bunolol OR Bupranolol OR butofilolol OR Butoxamine OR chlortalidone cloranolol OR cyanoiodopindolol OR carazolol OR carpindolol OR Carteolol OR Carvedilol OR Celiprolol OR cyanopindolol OR deacetylmetipranolol OR dexpropranolol OR diacetolol OR dichlorisoprenaline OR dilevalol OR Dihydroalprenolol OR diprafenone OR epanolol OR ersentilide OR esmolol OR exaprolol OR falintolol OR flusoxolol OR flestolol OR hydroxybenzylpinodolol OR hydroxycarteolol OR hydroxymetoprolol OR iodocyanopindolol OR iodopindolol OR iprocrolol OR isamoltane OR isoxaprolol OR indenolol OR levomoprolol OR Iodocyanopindolol OR Labetalol OR landiolol OR Levobunolol OR medroxalol OR mepindolol OR methylthiopropranolol OR moprolol OR mercuderamide OR Metipranolol OR Metoprolol OR Nadolol OR nevibolol OR nipradilol OR nifenalol OR oberadilol OR Oxprenolol OR pafenolol OR pamatolol OR primidolol OR procinolol OR pronetalol OR proxodolol OR Penbutolol OR Pindolol OR Practolol OR prizidilol OR Propranolol OR ridazolol OR salcardololOR Sotalol OR soquinolol OR spirendolol OR tazolol OR talinolol OR tienoxolol OR tolamolol OR toliprolol OR tribendilol OR tertatolol OR tilisolol OR Timolol OR trasitensin OR tobanum OR xibenolol OR zoleprodolol OR beta-Antagonist* OR beta-Adrenoceptor Antagonist* OR beta-Blocker* OR beta-Adrenergic Receptor Blockader* OR beta-Adrenergic Blocking Agent* OR beta Adrenergic Blocker* OR beta-Adrenergic Antagonist* OR Adrenergic beta-Receptor Blockader*):ti,ab,kw 6. (#1 OR #2 #3) AND (#4 OR #5)	(rosacea OR flush* OR (face OR facial) AND (erythem* OR redness)) AND ("adrenergic beta-Antagonist" OR Acebutolol OR adaprolol OR adimolol OR afurolol OR Alprenolol OR amosulalol OR arotinolol OR Atenolol OR befunolol OR bornaprolol OR brefonalol OR bucumolol OR bunitrolol OR Betaxolol OR bevantolol OR Bisoprolol OR bopindolol OR bromoacetylalprenololmenthane OR bucindolol OR bufetolol OR bufuralol OR Bunolol OR Bupranolol OR butofilolol OR Butoxamine OR chlortalidone cloranolol OR cyanoiodopindolol OR carazolol OR carpindolol OR Carteolol OR Carvedilol OR Celiprolol OR cyanopindolol OR deacetylmetipranolol OR dexpropranolol OR diacetolol OR dichlorisoprenaline OR dilevalol OR Dihydroalprenolol OR diprafenone OR epanolol OR ersentilide OR esmolol OR exaprolol OR falintolol OR flusoxolol OR flestolol OR hydroxybenzylpinodolol OR hydroxycarteolol OR hydroxymetoprolol OR iodocyanopindolol OR iodopindolol OR iprocrolol OR isamoltane OR isoxaprolol OR indenolol OR levomoprolol OR Iodocyanopindolol OR Labetalol OR landiolol OR Levobunolol OR medroxalol OR mepindolol OR methylthiopropranolol OR moprolol OR mercuderamide OR Metipranolol OR Metoprolol OR Nadolol OR nevibolol OR nipradilol OR nifenalol OR oberadilol OR Oxprenolol OR pafenolol OR pamatolol OR primidolol OR procinolol OR pronetalol OR proxodolol OR Penbutolol OR Pindolol OR Practolol OR prizidilol OR Propranolol OR ridazolol OR salcardololOR Sotalol OR soquinolol OR spirendolol OR tazolol OR talinolol OR tienoxolol OR tolamolol OR toliprolol OR tribendilol OR tertatolol OR tilisolol OR Timolol OR trasitensin OR tobanum OR xibenolol OR zoleprodolol OR beta-Antagonist* OR "beta-Adrenoceptor Antagonist*" OR beta-Blocker* OR "beta-Adrenergic Receptor Blockader*" OR "beta-Adrenergic Blocking Agent*" OR "beta Adrenergic Blocker*" OR "beta-Adrenergic Antagonist*" OR "Adrenergic beta-Receptor Blockader*")
Until 20-11-2019: **124 hits**	Until 21-11-2019: **120 hits**

Table S2. In –and exclusion criteria.

Inclusion criteria	**Exclusion criteria**
• Cutaneous rosacea with flushing and/or persistent facial erythema • Oral β-blockers • Adolescents and adults (≥ 16 years) • Randomized controlled trials, non-randomized controlled trials, cohort studies, case-control studies, case series, case reports	• Ocular, extrafacial and drug-induced rosacea • Drug-induced flushing • Topical β-blockers (brimonidine) • Children (< 16 years) • In vitro and animal studies • Languages other than English, German or Dutch • Meta-analysis systematic reviews and reviews (no primary source) • Full-text not available • Abstracts of congresses • Duplicates

Table S3. Review author's judgement about each risk of bias item for the included case-control study (*n*=1) with the Newcastle-Ottawa scale.

	Selection				Comparability		Exposure			Quality score
Author + year	**Adequate case definition**	**Representativeness of cases**	**Selection of controls**	**Definition of controls**	**Comparability of cases and controls for important factor (sex)**	**Comparability of cases and controls for additional factors (age etc)**	**Exposure ascertainment**	**Same method of ascertainment for cases and controls**	**Non-response rate**	
Spoendlin 2014	★	★	★	★	★	★	★	★		8

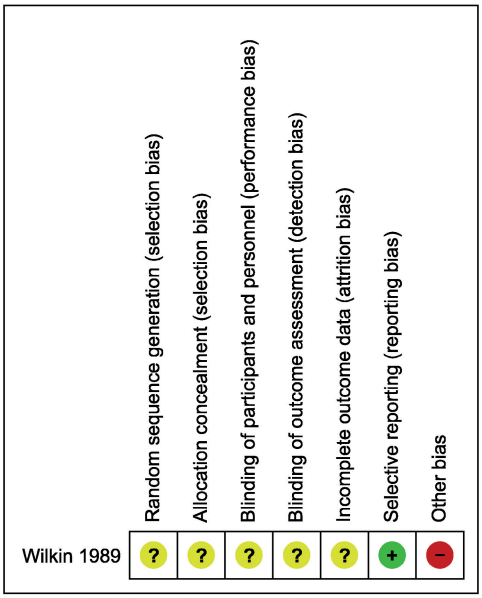

Figure S1. Review author's judgement about each risk of bias item for the included RCT (*n*=1) with the Cochrane Risk of Bias tool.

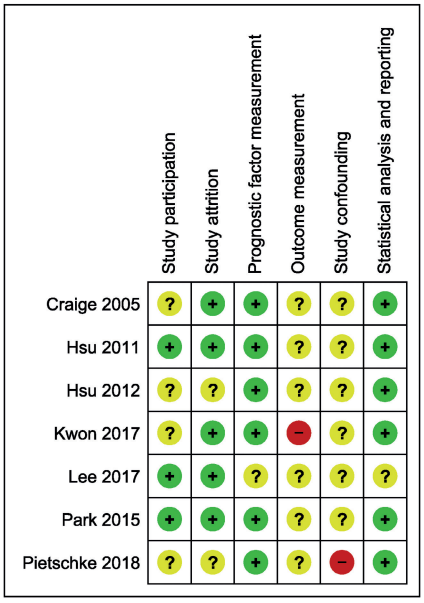

Figure S2A. Review author's judgement about each risk of bias item for each included cohort study and case report/series (*n* = 7) with the Quality in Prognosis Studies tool.

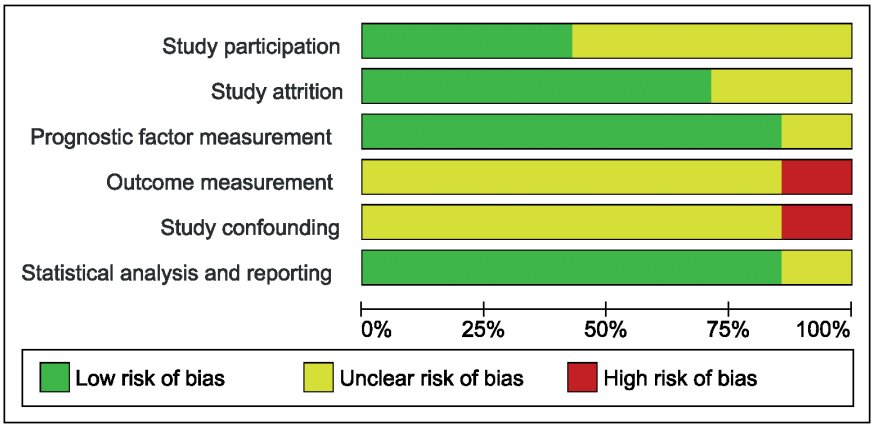

Figure S2B. Review author's judgement about each risk of bias item presented as percentages across all included cohort studies and case reports/series (*n* = 7) with the Quality in Prognosis Studies tool.

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CHAPTER 4

Summary and general discussion

THESIS SUMMARY

The aim of this thesis was to evaluate the value of novel and conventional noninvasive imaging and biophysical tools in healthy and inflamed skin in daily clinical practice, with emphasis on rosacea. Rosacea was chosen because it is localized at a visible and cosmetically important region (the face), it has variable signs and symptoms which makes objective clinical evaluation extra challenging, and it responds well to anti-inflammatory treatment. In this final chapter the main findings of the studies described will be summarized and discussed on the basis of the following two main objectives:

1. To investigate the value of imaging and biophysical tools in healthy and impaired skin barrier function.
2. To investigate the value of imaging and biophysical methods in inflamed skin, with emphasis on rosacea.

Table 1 provides a summary of research questions, study designs, and assessed parameters per study included in this thesis.

Table 1. Overview of methods used per study included in this thesis.

Chapter	Research question	Study design	Parameters assessed
2.1	To determine the use of the Epsilon® in the measurement of anatomical site variation of water content in the skin	• Explorative, prospective study • Data comparison with current knowledge (literature)	• Water content, TEWL
2.2	To investigate the feasibility of combining four existent biophysical/imaging devices to measure differences in skin barrier function after cream application	• Explorative, prospective study	• Erythema, water content, TEWL, skin thickness
2.3	To determine the value of the GPSkin® to measure skin barrier impairment and to monitor barrier function in rosacea in daily practice	• Explorative, prospective study (2 pilots) • Tapestripping	• Clinical scores • Water content, TEWL
3.1	To provide an extensive overview of available noninvasive objective skin measurement techniques for rosacea assessment	• Literature • Narrative review	• RCM, dermatoscopy, capillaroscopy, OCT, computer-aided image analysis, infrared photography, sonography • Water content, TEWL, sebum, pH, erythema, skin blood flow, skin temperature
3.2	To examine the value of RCM for the monitoring of rosacea during treatment with topical ivermectin	• Explorative, prospective study • RCM image analysis • Mixed model logistic regression analysis	• Clinical scores • RCM parameters (*Demodex*, inflammation, vascularization)
3.3	To develop and evaluate an image-based erythema quantification tool to monitor facial erythema in rosacea patients during treatment	• Prospective data acquisition • Retrospective image analysis (ImageJ®) • Interobserver correlation	• Photographs, clinical scores • Quantitative colour calculation (RGB; red/green ratio, CIELAB: a^* and Δa^*)
3.4	To present five rosacea patients with worsening of symptoms due to occlusion of the skin after use of a CPAP mask	• Retrospective case series	• Clinical data about OSAS and rosacea
3.5	To evaluate the efficacy of oral beta-blockers in rosacea-associated erythema and facial flushing	• Literature • Narrative review	• Erythema, facial flushing

OCT, optical coherence tomography. RCM, reflectance confocal microscopy. TEWL, transepidermal water loss

Aim 1. To investigate the value of imaging and biophysical tools in healthy and impaired skin barrier function.

The normal human skin barrier prevents excessive water loss and dehydration of our body. The main barrier function of the skin is formed by the stratum corneum (SC). In case of a disrupted skin barrier, inflammation can occur due to production of pro-inflammatory cytokines and proliferation of keratinocytes. An impaired skin barrier function is seen in several chronic inflammatory skin disorders, such as ichthyosis vulgaris, atopic dermatitis, plaque psoriasis, and possibly also rosacea. Skin barrier function can be assessed by objective determination of transepidermal water loss (TEWL) and water content.

In **chapter 2** we evaluated the value of novel and combined biophysical tools to assess normal and impaired skin barrier function. First, we investigated the Epsilon® to determine its use in the measurement of anatomical site variation of water content in the healthy skin (**chapter 2.1**). The Epsilon® is a novel device which measures water content with multiple electronic sensors. Using the Epsilon®, we found significant differences in water content in various anatomical regions: the cheek had the highest water content, followed by the forearm, abdomen, lower back, and mid-calf. We discovered that the Epsilon® consistently measures lower water content values compared to single sensor corneometers. The Epsilon® may be more accurate in measuring water content values in the stratum corneum compared to single sensor devices for three reasons. First, due to its multisensory character and small sensors, measurements are restricted to the 'dry' SC only. Second, it corrects for time-dependent skin occlusion differences. Third, multiple simultaneous measurements can be averaged or used to get a hydration overview image of the SC (*skin mapping*). By accurately measuring water content values, it is possible to precisely determine the degree of skin barrier disruption in inflammatory skin diseases, which enables easy monitoring of treatment effects. Disadvantages of the Epsilon® are its start-up time (approximately 10 minutes) and the requirement of a wired connection to a laptop, limiting its portability. Therefore, rapid use in clinical practice is difficult.

In **chapter 2.2** we focused on the feasibility of four different biophysical/imaging devices to assess the healthy skin barrier function before and 30 minutes after application of Lanette or petrolatum cream. Erythema was determined by spectrophotometry (i.e. quantitative measurement of the reflection of visible light as a function of wavelength), water content by the Epsilon®, transepidermal water loss (TEWL) by the Aquaflux device (a device with a probe that calculates water vapor flux due to diffusion), and epidermal thickness by reflectance confocal microscopy (RCM, a noninvasive imaging technique for *in vivo* visualization of the superficial skin layers with a resolution comparable to conventional histology). We found that SC thickness was significantly increased after application of both creams. Erythema, TEWL, and water content did not significantly change after cream application. Our multiparametric approach demonstrates a feasible way to quickly obtain multifaceted information about the skin

barrier function, which could not have been obtained by a single device only. In an ideal world, for reasons of convenience this multiparametric outcome would be produced by one single, small, and wireless device. Our multiparametric approach provides very detailed information about the status of the skin barrier function in inflammatory diseases, both before and during treatment, allowing individualized treatment options. Furthermore, it may enable better understanding of the individual pathophysiological processes that are be responsible for cutaneous damage and subsequent restoration. Some disadvantages of our multiparametric approach are the need for a spacious room (especially the RCM is quite bulky), and the separate protocols for each device.

In **chapter 2.3** we determined the value of the GPSkin® (a novel, portable handheld device that measures water content and TEWL simultaneously) to assess impaired skin barrier function after tapestripping, which is a procedure to mechanically remove the SC using tapestrips. Moreover, we also investigated its usability for monitoring skin barrier function at the cheeks of rosacea patients before and during treatment in daily practice. We found a strong and linear correlation between water content/TEWL values of the new device (the GPSkin®) and the other devices (the Aquaflux® and Epsilon®), both before and after tapestripping. Water content was significantly lower at the cheeks of rosacea patients compared to controls, with a trend towards recovery during successful anti-inflammatory treatment. TEWL was comparable between patients and controls, and did not change during treatment. Compared to the other skin barrier tools, the GPSkin® is much more usable in clinical practice due to the small size, wireless character, and rapid mode of action. Moreover, in contrast to the other devices, water content and TEWL are measured at exactly the same location and time, preventing probe replacements compared to using two separate devices. The latter is essential for accurate temporal measurements at the face, because of large water content and TEWL differences within very small distances on the face due to various environmental influences such as sebum gland activity and cutaneous vasculature.

Main conclusions for aim 1:

- The Epsilon® is able to measure significant differences in water content in various anatomical locations. Compared to conventional water content devices, Epsilon® results are likely more accurate.
- A multiparametric approach using the Epsilon®, Aquaflux®, spectrophotometer and RCM, to measure both water content, TEWL, erythema, as well as epidermal thickness, offers a practical way to obtain multifaceted information about skin barrier function.
- The GPSkin® is a very practical tool to determine TEWL and water content in an accurate, simple, and rapid manner, both in normal skin as well as in skin with impaired barrier. We were able to measure improvement of skin barrier function in rosacea during successful treatment in daily practice.

Aim 2. To investigate the value of imaging and biophysical tools in inflamed skin, with emphasis on rosacea.

Rosacea is a chronic inflammatory facial skin disease of uncertain etiology, characterized by among others papules, pustules, erythema, telangiectasia, and flushing. It is an interesting inflammatory skin disease for investigation of noninvasive techniques, due to its facial localization (making invasive techniques less attractive), chronic character with exacerbations and remissions (allowing temporal monitoring), multifactorial pathophysiology, wide range of clinical symptoms, and good response to successful anti-inflammatory treatment. Its current diagnosis is largely based on clinical evaluation, which is subjective and prone to interobserver variation. This makes treatment effect evaluation and comparison of study outcomes challenging.

The purpose of **chapter 3** was to investigate the value of novel and conventional imaging and biophysical tools in rosacea for use in daily clinical practice. In summary, we tried to correlate objective imaging and biophysical parameters with clinical symptoms. This could result in obtaining a more objective measurement of the current status of the (inflamed) skin.

Biophysical and imaging tools have already extensively been used in rosacea, but a clear overview was lacking. In **chapter 3.1**, we conducted a systematic review of available noninvasive objective skin measurement techniques for diagnosis, assessing severity and therapy monitoring of rosacea. An extensive literature search revealed 78 eligible studies. Imaging techniques used were RCM, dermatoscopy, capillaroscopy, optical coherence tomography, computer-aided image analysis, infrared photography, and sonography. Assessed biophysical parameters were SC hydration, TEWL, sebum excretion, pH, erythema, skin blood flow, and skin temperature. Most of the included studies were cohort studies, followed by case-control studies, randomized controlled trials, case reports, and case series. Quality of included studies was regularly low due to high risk of various forms of bias, small sample sizes, large interstudy outcome variability, and often a lack of method standardization. Despite these limitations, several tools showed additional value in monitoring rosacea. For treatment follow-up, *Demodex* mites were easily counted by RCM, erythema was accurately monitored with spectrometry, and rosacea severity was quantified by skin hydration and TEWL measurements. We recommended developing adequate and validated protocols for further implementation of these tools in the research setting.

In **chapter 3.2** we examined the value of RCM for the monitoring of rosacea during a 16-week treatment course with topical ivermectin. Treatment resulted in a clinically significant reduction of inflammatory lesions and erythema, but telangiectasias were not reduced. A clear decrease in number of *Demodex* mites inside the hair follicles was measured using RCM. However, *Demodex* was often difficult to distinguish from other cellular structures such as sebum or hairs. No clear changes in inflammatory

cells, epidermal thickness, vascular diameter, and vascular density were observed. The correlation between the number of inflammatory lesions and *Demodex* mites was low, and none of the RCM variables were significant predictors for clinical success. Therefore, the RCM in its current form seems of limited value for monitoring of rosacea patients in daily practice. Our results are most likely explained by various RCM device limitations, which will be described later on in this chapter.

In **chapter 3.3** we developed and evaluated an image-based erythema quantification method using ImageJ® software to monitor facial erythema in rosacea patients during treatment with topical ivermectin. For this, we used standardized clinical photographs of the patient's face, the RGB (red-green-blue) color model, and CIELAB colour space model (*a**, indicating colour from green to red, corresponding well to the observation of the human eye). We found no significant changes in the RGB model values, but *a** significantly decreased during successful treatment of rosacea. A weak correlation existed between clinical scores and lesional *a**. Interobserver correlation was high. ImageJ® is a very simple, rapid, and open-source imaging software which can be easily used by clinicians. The photographs allow retrospective analysis, mapping of large and small anatomical locations, follow-up of exactly the same location, discrimination of subtle redness differences, and does not interfere with skin colour due to its non-contact character. As erythema is an important feature of inflammation, standardized clinical images in combination with ImageJ® can improve objective measurement of inflammatory skin diseases (e.g. rosacea, psoriasis, and atopic dermatitis) compared to visual inspection.

In **chapter 3.4**, we reported five patients with development or worsening of rosacea symptoms after use of a full-face continuous positive airway pressure (CPAP) mask covering nose and mouth for obstructive sleep apnea syndrome (OSAS). These patients were diagnosed with papulopustular rosacea, erythematotelangiectatic rosacea, morbus Morbihan, and/or rhinophyma. Three patients had nasal skin symptoms and two patients showed centrofacial symptoms consistent with the shape of the CPAP mask. We propose an occlusive effect of the CPAP mask on the skin, increasing skin humidity, skin sebum, skin temperature, and in the gut of *Demodex* mites possibly also bacterial activity, inducing primary symptoms of an underlying sensibility for rosacea. It would be interesting to further examine our hypothesis by measuring these parameters objectively in OSAS patients before and during CPAP treatment. This could lead to profound insights into the mechanisms behind rosacea development in this population.

Lastly, in **chapter 3.5**, the efficacy of oral beta-blockers for rosacea-associated facial flushing and erythema was evaluated by performing a systematic review of the available literature. In total, nine studies were included. We discovered that it was difficult to compare individual study outcomes due to a large interstudy outcome variability. Erythema and flushing were assessed using a wide spectrum of subjective and

objective parameters such as clinical and patient scoring, clinical photographs, laser Doppler, skin temperature, and dermoscopy. Standardized and objective assessment of erythema and flushing would greatly facilitate the comparison of individual studies, which may lead to more solid conclusions about the effect of a particular treatment.

Main conclusions for aim 2:

- Multiple imaging and biophysical tools have yet been tested for rosacea assessment. RCM, spectrophotometry, and determination of water content and TEWL seem promising to monitor rosacea. Our systematic review shows the need for larger, standardized studies.
- RCM enables anti-inflammatory effect monitoring by determining mite presence, but quantifying exact mite number and inflammatory and vascular parameters is challenging due to device limitations. RCM seems of limited value for follow-up of rosacea in clinical practice.
- ImageJ® software supports simple, rapid, objective, and reproducible quantification of facial erythema. The standard medical photographs allow retrospective analysis, evaluation of large and small lesions, and discrimination of subtle redness differences.
- We hypothesize that CPAP masks for OSAS can induce rosacea symptoms due to skin occlusion, increased skin temperature and humidity, and activation of *Demodex*-related bacteria. Investigation of this hypothesis may lead to additional insights in the pathophysiology of rosacea.
- It is difficult to compare outcomes of individual studies about facial erythema and flushing, due to a large heterogenicity in determination of these parameters. Objective evaluation of erythema and flushing would lead to easier comparison of these study results.

Rosacea as a model for skin inflammation

We consider rosacea to be a suitable model for monitoring inflammatory diseases, because it is localized at a visible and cosmetically important region, it has variable signs and symptoms which are making objective clinical evaluation challenging, and it responds well to effective anti-inflammatory treatment. However, our findings after extensive experience show that the rosacea model may have some restrictions. As outlined above, the facial region has many convex areas and sharp corners, which makes application of contact tools challenging. Additionally, skin lesions (i.e. erythema, inflammatory papules and pustules, telangiectasia) in rosacea often have a diffuse character, complicating the distinction with non-lesional facial skin. Furthermore, facial skin shows large skin barrier differences within very small distances due to internal and environmental influences such as variation in sebum gland density, skin pH, humidity, skin type, UV light, and air pollution.[1-3] Before implementation, it is therefore important to investigate the tools examined in this thesis in other inflammatory diseases, both with facial and non-facial clinical symptoms, as well.

Benefits of noninvasive tools

Until today, diagnosis and evaluation of inflammatory skin conditions is mainly based on visual inspection. However, recognizing subtle changes in skin symptoms is extremely challenging by visual assessment only. The image perceived by the human eye is a complex and nonlinear comprehension, being qualitative and individual. This makes visual evaluation subjective and prone to interobserver variation. From a patients' point of view, visual scores are difficult to understand and interpret. Moreover, visual evaluation does not provide information about subsurface skin processes, while the most important early events in the pathogenesis of dermatoses are hidden beneath the skin surface. Visible symptoms occur at a later disease stage. Additionally, resolving of clinical symptoms is not equal to complete disease remission. For example, in atopic dermatitis, healthy-looking skin has a disrupted skin barrier function,[4] and cytokine and T-cell activity is still upregulated in uninvolved skin in patients with psoriasis.[5] Noninvasive assessment of skin parameters provides qualitative and quantitative, objective, and reproducible information about the anatomy, function, and properties of the interior of the skin which cannot be collected by human visual observation alone. Imaging techniques such as RCM are suitable to gain additional information about subsurface skin morphology and inflammatory processes such as dynamics of inflammatory cells, vascularisation, and *Demodex* mites. This increases our knowledge about how dermatoses evolve and resolve at the subclinical level. Noninvasive techniques might reveal that apparently cleared skin sites are still inflamed, or may identify invisible early stage disease before the appearance of clinical symptoms, allowing to start effective treatment in a very early stage. This may prevent extended disease and irreversible skin damage.

Compared to histological evaluation of a skin biopsy, noninvasive techniques enable repeated monitoring of the same skin site over time without causing discomfort, pain, damage or scarring, which is especially important in cosmetically important regions such as the face. Additionally, biopsies provide a static picture at a specific point in time and interfere with the integral skin structure, while by using noninvasive tools the native skin structure is not altered, prohibiting challenges in result interpretations. Moreover, especially in resource limited settings, diagnostic devices could potentially reduce the delay in obtaining -expensive- histopathologic diagnosis.

For the research setting, objective assessment of cutaneous parameters would greatly improve comparison of individual study outcomes. Nowadays, for evaluation of many inflammatory dermatoses a golden standard is lacking, resulting in a mixture of parameters being assessed. Universal, objective assessment enables proper evaluation of treatment effects in systematic reviews and meta-analysis. In this way, firmer conclusions can be drawn about the actual effects of treatments. This would help in developing more robust, evidence-based treatment guidelines.

Hurdles

While having many advantages, there are still hurdles to be overcome before noninvasive devices for evaluation of inflammatory dermatoses can be implemented in daily clinical practice. These obstacles are due to various technical and practical factors, which will be discussed in more detail in the next paragraph.

Many of the available noninvasive techniques are expensive, not commercially available, and highly specialized and only useful for investigators with a specific focus and training. Current tools can measure only one or a few parameters in the very complex environment of the skin.[6] Contact devices may interfere with skin colour due to vessel compression. Biophysical tools provide point measurements (usually in the range of cm^2), questioning representativeness of the results for an entire region. Repeated measuring on exactly the same location is challenging with a small probe. On the other side, larger probe heads will prevent measurements in recessed body parts such as the nose. Larger devices (e.g. the RCM, but also other noninvasive imaging methods which have been tested for skin imaging such as ultrasound, magnetic resonance imaging, nuclear magnetic resonance spectroscopy, near infrared imaging, optical coherence tomography, and Raman spectroscopy[7-11]) have some specific disadvantages. Due to their cost, size, weight, non-wireless character, and start-up time, their practical use is limited. Imaging of convex areas such as the eye region and nose fold is impossible, and extensive training about skin morphology may be needed to adequately interpret image results. Additionally, image depth and/or resolution may be limited. Images are often two-dimensional, horizontal to the skin surface (instead of vertical sections corresponding to histology) and in black and white only. Just recently, a low-cost, portable RCM connected to a smartphone was being developed;[12] this could be a very promising upgrade compared to the current, bulky device. Three-dimensional imaging would be a very helpful improvement to retrieve optimal architectural and cellular information, and may also aid in quantifying elevated -inflammatory- lesions at the skin surface.[9] 3D imaging has already been used for evaluation of scars, such as atrophic acne scarring and keloids.[13,14] The VISIA® Skin Analysis System is a commercially available device with quantitative facial imaging analysis software, primary used for aesthetic and skin care consultations. It uses multi-spectral imaging (standard, UV, and polarized light) and analysis to capture visual information with respect to areas that affect the health and look of the skin from a cosmetic point of view: visible spots, UV spots, brown spots, wrinkles, texture, pores, red areas, and porphyrins (i.e. bacterial presence in pores).[15-17] However, these outcome measures are not suitable as diagnostic features, because most of them are not directly related to inflammatory processes. Moreover, despite having fine image quality, this system is not very accurate for skin mapping due to software limitations, and the device is large, expensive, not easily portable, and designed for facial analysis only.[17]

Beside from techniques aspects, one must be aware of the occurrence of various internal and external human variations when using noninvasive devices. Determination

of TEWL, SC hydration and erythema may be influenced by skin blood flow, pH, sebum gland density, anatomical location, skin surface and environmental temperature, sweating, skin damage, air humidity, topical application of products, skin type, and sun exposure.[1,18-20] It is therefore important to interpret the results in the light of these possible factors. Considering the possible intra-individual variation of skin parameters, we recommend to always register a baseline value or a reference untreated control site (i.e. non-lesional skin).[6]

Future perspectives: the ideal tool

In order to be suitable for use in daily clinical practice, a device for evaluating inflammatory skin diseases should be time *saving*, instead of time *consuming*. In daily practice, measurement according to extensive guidelines with standardization of environmental circumstances is therefore far from feasible.[6] Based on the current knowledge and the results of this thesis, we assembled a core set of properties that the 'ideal tool' for assessment of inflammatory skin diseases should comply with (Table 2). This ideal tool provides a core set of skin parameters, providing an overall view of current objective status of the (inflamed) skin.[21]

In this thesis, we showed that TEWL, water content, and erythema determination are promising objective parameters for the evaluation of inflamed skin. To determine which core set of parameters should be measured by the 'ideal tool', features other than the ones examined in this thesis must be explored. Sebum, skin pH, natural moisturizing factor (NMF),[6,22-30] and biomarkers such as cutaneous cytokines may be interesting targets.[30-39] Further research is needed to determine which parameters should be integrated in this core set of parameters, focussing on their intended purpose to monitor the severity of inflammation, sensitivity, specificity, and cost-effectiveness.[40-42]

To facilitate result interpretation, automated analysis using machine learning and artificial intelligence is a promising perspective. This may overcome low reproducibility of some parameters, and reduce precious analysis time. Machine learning has already been applied in various medical specialties such as radiology,[43] pathology,[44] orthopaedics,[45] internal medicine,[46] and, although still in its infancy, also in dermatology.[47-49] We expect that increasingly sophisticated computer-assisted techniques will find its way into dermatological practice. However, an expert should oversee and double-check the process of (automatic) integrating of all available information to form a well-considered diagnosis and treatment regimen.

Table 2. Characteristics of the ideal noninvasive tool for evaluation and follow-up of inflammatory skin diseases in daily practice.

- Portable, wireless, light-weight, pocket-sized; or human-sized (like magnetic resonance imaging)
- Non-contact
- Affordable
- Commercially available
- Rapid measurement (< 5 min)
- No need for repeated calibration
- Easy to use, directly operational without start-up time
- Provides distribution map for entire region of interest
- Insensitive to external factor influences
- Immediate reading and interpretation of results for direct feedback to the patient
- Options for automatic retrospective analysis at the computer using a simple software package
- Combination of core set of biophysical and imaging measurements, which are measured simultaneously
- Surface and subsurface skin parameters
- 3D images with sufficient penetration until subcutis

Final statements

Overall, in this thesis the value of novel and existing noninvasive imaging and biophysical tools in healthy and inflamed skin in daily practice was outlined. By means of this thesis, we tried to encourage clinicians as well as researchers to further investigate and improve the application of biophysical and imaging devices in the clinical and experimental dermatological setting. Once the most important hurdles have been overcome, a widespread application of these techniques in dermatological practice can be expected. We hypothesize in the future inflammatory skin diseases will be monitored with an 'ideal tool', replacing subjective scoring of severity of inflammation. This could greatly improve objective skin disease severity assessment in the clinical context, and promotes harmonization of outcomes in a research setting. Ideally, every patient with an inflammatory skin disease will possess the 'ideal tool' for home-based skin monitoring, enhancing patient involvement, personalized treatment, and therapy compliance.

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CHAPTER 5

Nederlandse samenvatting

Onze huid vormt een belangrijke eerste barrière tegen potentieel schadelijke invloeden van buitenaf zoals bacteriën, chemicaliën, ultraviolet (UV) licht, en mechanische schade. Ook voorkomt de huidbarrière overmatig waterverlies via de huid. De huidbarrière wordt voornamelijk gevormd door de buitenste huidlaag, het stratum corneum (SC). De staat van de huidbarrière is objectief te meten met niet-invasieve apparatuur die de morfologie van de huid in kaart brengt (beeldvorming, Engels: '*imaging*'), en die de functie van de barrière meet middels biofysische huidkenmerken (bijv. transepidermaal waterverlies [TEWL], waterinhoud, roodheid). Indien de huidbarrière verstoord is, kan de huid uitdrogen en kan er tevens ontsteking in de huid ontstaan. Een verstoorde barrière kan het gevolg zijn van mechanisch trauma, maar wordt ook gezien bij diverse ontstekingsziekten van de huid, zoals eczeem, psoriasis, en mogelijk ook rosacea. Het doel van dit proefschrift was het evalueren van nieuwe en bestaande niet-invasieve beeldvormende en biofysische methoden in gezonde en ontstoken huid in de dagelijkse klinische praktijk, met focus op rosacea. In het eerste deel (**hoofdstuk 2**) wordt de waarde van biofysische en beeldvormende meetmethoden in gezonde huid en huid met een verminderde huidbarrière onderzocht; in het tweede deel (**hoofdstuk 3**) wordt hun waarde in ontstoken huid bekeken, met focus op rosacea. Rosacea is een inflammatoire huidaandoening van het gelaat. Omdat juist in het gelaat niet-invasieve meetmethoden te prefereren zijn boven invasieve, werd de waarde van deze methodiek bij rosacea onderzocht.

Doelstelling 1. Het onderzoeken van de waarde van beeldvormende en biofysische technieken bij gezonde en verstoorde huidbarrière.

In **hoofdstuk 2.1** onderzochten we de waarde van de Epsilon®, een meetinstrument dat middels een array van sensoren de waterinhoud van de huid meet, gecombineerd met een morfologisch beeld van het stratum corneum (SC) op basis van de variatie in het gemeten watergehalte. We vonden significante verschillen tussen waterinhoud van het SC van verschillende anatomische regio's; de wang had de hoogste waterinhoud, gevolgd door de onderarm, abdomen, rug, en kuit. Ook vonden we dat de Epsilon® consistent lagere waardes meet vergeleken met conventionele, vergelijkbare tools die maar één sensor hebben. Dit kan verklaard worden doordat de Epsilon® waterinhoud nauwkeuriger meet dan conventionele tools; de meting blijft beperkt tot het SC, het apparaat corrigeert voor tijdsafhankelijke occlusie-effecten, en er vinden meerdere metingen tegelijkertijd plaats die gebruikt kunnen worden voor een 'hydratie-map'. Hierdoor is het mogelijk om de mate van verstoring van de huidbarrière in ontstekingsziekten van de huid nog nauwkeuriger te bepalen. Dit vergemakkelijkt het monitoren van behandeleffecten. Enkele nadelen van de Epsilon® zijn de opstarttijd (ca. 10 minuten), en de noodzaak tot kabelverbinding met een laptop, waardoor de draagbaarheid beperkt is. Hierdoor lijkt het meetinstrument in de dagelijkse praktijk nog minder goed bruikbaar.

In **hoofdstuk 2.2** focusten we ons op het combineren van vier verschillende biofysische en beeldvormende apparaten om de huidbarrièrefunctie te meten zowel vooraf als 30 minuten na applicatie van twee soorten emollientia (lanette crème en vaseline-lanette crème). Erytheem werd gemeten via spectrofotometrie (i.e. kwantitatieve bepaling van de reflectie van zichtbaar licht als een functie van de golflengte), waterinhoud met de Epsilon®, transepidermaal waterverlies (TEWL) met de Aquaflux® (een apparaat met een probe dat de waterdampstroom door diffusie vanuit de huid naar de omgeving meet) en dikte van het SC met de reflectie confocale microscoop (RCM; een niet-invasieve imaging techniek voor het *in vivo* visualiseren van de bovenste huidlagen met een resolutie die vergelijkbaar is met conventionele histologie). Wij vonden een significant grotere dikte van het SC ná het smeren van beide crèmes vergeleken met daarvoor. Erytheem, TEWL, en waterinhoud veranderden niet na applicatie van crème. Met onze multi-parameter aanpak is het mogelijk om veelzijdige informatie over de staat van de huidbarrière in ontstekingsziekten van de huid te verzamelen, zowel voor als na een behandeling. Idealiter leidt dit tot een beter begrip van etiologische processen die betrokken zijn bij huidschade -en herstel, en gepersonaliseerde behandelopties. Om de praktische toepasbaarheid te vergroten zouden deze metingen bij voorkeur niet door vier losse tools, maar door één en hetzelfde apparaat uitgevoerd worden dat bovendien klein en draadloos is. Enkele nadelen van onze multiparameter-aanpak zijn de noodzaak van een ruime onderzoekskamer en de separate protocollen behorende bij elke device.

Vervolgens bepaalden we in **hoofdstuk 2.3** de waarde van de GPSkin®, een apparaat dat waterinhoud en TEWL tegelijk meet, om een defecte huidbarrière te meten. De huidbarrière werd middels tapestripping verstoord, een onderzoeksmodel waarbij het SC mechanisch verwijderd wordt middels herhaaldelijk aanbrengen van plakband. Ook onderzochten we de bruikbaarheid van de GPSkin® voor het monitoren van de barrièrefunctie bij patiënten met rosacea vóór en tijdens behandeling in de dagelijkse praktijk. We vonden een sterke lineaire correlatie tussen waterinhoud/TEWL-waardes van de GPSkin® en conventionele apparatuur (de Epsilon® en Aquaflux®), zowel vóór als na tapestrippen. De waterinhoud van de huid van rosaceapatiënten was significant lager dan die van proefpersonen met gezonde huid, waarbij er een trend was naar herstel gedurende succesvolle anti-inflammatoire behandeling middels topicaal ivermectine, een reeds effectief gebleken crème voor inflammatoire laesies bij rosacea (zie **hoofdstuk 1** voor de behandelopties bij rosacea). TEWL was vergelijkbaar bij rosaceapatiënten en controles, en veranderde ook niet tijdens behandeling. Vergeleken met andere beschikbare instrumenten die de huidbarrière meten is de GPSkin® veel gebruiksvriendelijker in de dagelijkse praktijk door zijn handzame formaat, draadloze karakter, en snelheid. Bovendien worden waterinhoud en TEWL op exact dezelfde plek en tijd gemeten. Dit is extra belangrijk in het gelaat, waar grote verschillen in deze parameters bestaan binnen zeer kleine afstanden (centimeters).

Hoofdconclusies doelstelling 1:

- De Epsilon® is in staat om significante verschillen in waterinhoud te meten op verschillende anatomische locaties. Vergeleken met conventionele apparaten die waterinhoud meten, meet de Epsilon deze waarden nauwkeuriger.
- Een multi-parameter aanpak waarbij de Epsilon®, Aquaflux®, spectrofotometer, en reflectie confocale microscoop worden gebruikt om respectievelijk waterinhoud, TEWL, erytheem, en epidermale dikte te meten, biedt een praktische en kwantitatieve manier om veelzijdige informatie te vergaren over de staat van de huidbarrière.
- De GPSkin® is een zeer praktische tool om TEWL en waterinhoud op een nauwkeurige, simpele, en snelle manier te bepalen, zowel bij een normale als een verstoorde huidbarrière. We waren in staat om verbetering van de huidbarrièrefunctie te meten tijdens succesvolle behandeling van rosaceapatiënten in de dagelijkse praktijk.

Doelstelling 2. Het onderzoeken van de waarde van beeldvormende en biofysische technieken in de ontstoken huid, met focus op rosacea.

Rosacea is een chronische ontstekingshuidziekte van het gelaat, met een scala aan symptomen, waaronder papels, pustels, erytheem, en teleangiëctasieën. De etiologie is tot op heden onopgehelderd, maar lijkt multifactorieel (immunologisch, neurovasculair, externe en genetische factoren, *Demodex* mijten, huidbarrière). De ernst van rosacea wordt momenteel vastgesteld door klinische symptomen te scoren. Dit is subjectief en gevoelig voor interobserver-variabiliteit. Hierdoor is het lastig om behandeleffecten te bepalen en om studie-uitkomsten onderling te vergelijken. Het afnemen van een huidbiopt is invasief en kan littekens achterlaten, wat zeker in het geval van een huidziekte in het gelaat niet wenselijk is. Derhalve is het ziektebeeld rosacea zeer bruikbaar voor onderzoek naar niet-invasieve meetmethoden van de huid.

Om rosacea te evalueren zijn er diverse biofysische en beeldvormende tools toegepast, maar een duidelijk overzicht van deze tools ontbrak tot op heden. In **hoofdstuk 3.1** werden op systematische wijze de reeds onderzochte niet-invasieve meetmethoden voor de diagnostiek en follow-up van rosacea in kaart gebracht. In deze studie werden 78 studies geïncludeerd. Gebruikte beeldvormende technieken waren reflectie confocale microscopie, dermatoscopie, capillaroscopie, optische coherentie tomografie, computergestuurde beeldanalyse, infrarood fotografie, en echografie. Biofysische parameters die werden bepaald waren waterinhoud, TEWL, sebumexcretie, pH, erytheem, cutane doorbloeding, en huidtemperatuur. Helaas was de kwaliteit van de studies laag door kleine patiëntaantallen, een grote variabiliteit in studie-uitkomsten, en een gebrek aan standaardisatie van de gebruikten meetmethoden. Desondanks lijken reflectie confocale microscopie, spectrofotometrie en bepaling van waterinhoud en TEWL veelbelovende technieken om de effecten van de behandeling van rosacea te monitoren.

Vervolgens werd in **hoofdstuk 3.2** de waarde van reflectie confocale microscopie voor het monitoren van rosacea onderzocht tijdens een 16 weken durende behandeling met topicaal ivermectine. Behandeling resulteerde inderdaad in een afname in aantal klinische inflammatoire huidafwijkingen. Middels RCM werd vastgesteld dat het aantal *Demodex* mijten in de haarfollikels op de wangen van de patiënten met rosacea significant afnam. Echter, het bleek lastig om *Demodex* mijten op basis van RCM beeld te onderscheiden van bijv. talg of haren. Geen evidente veranderingen werden middels RCM geobserveerd in aantal ontstekingscellen, epidermale dikte, en vaatdiameter -en dichtheid. De correlatie tussen het aantal inflammatoire huidafwijkingen en het aantal *Demodex* mijten was laag, en geen enkele RCM-parameter bleek een significante voorspeller voor klinische verbetering. Derhalve werd geconcludeerd dat de RCM in zijn huidige vorm van beperkte waarde lijkt voor het monitoren van rosacea in de dagelijkse praktijk. Dit hangt met name samen met diverse beperkingen van het RCM apparaat, zoals de beperkte penetratiediepte in de huid, het zwart-wit en tweedimensionale karakter van de afbeeldingen, en de noodzaak tot uitgebreide training van de onderzoeker voor interpretatie van de afbeeldingen.

In **hoofdstuk 3.3** werd een objectieve methode om klinisch erytheem (roodheid) te kwantificeren ontwikkeld en geëvalueerd. Erytheem is een belangrijke uiting van inflammatie. Wij monitorden het erytheem in het gelaat van rosaceapatiënten voor, tijdens, en na behandeling met topicaal ivermectine. Met behulp van ImageJ® software werden klinische, gestandaardiseerde foto's van patiënten geanalyseerd en werd het erytheem beoordeeld binnen het CIELAB kleurenspectrum model. Hierbij weerspiegelt de waarde van *a** de mate van roodheid, corresponderend met de observatie van het menselijk oog. Wij toonden aan dat de *a**-waarde significant daalde tijdens succesvolle anti-inflammatoire behandeling van rosacea. Een zwakke relatie werd gevonden tussen klinische scores en *a**. De interobserver-correlatie voor het berekenen van *a** was hoog. ImageJ is een zeer eenvoudig, snel, en vrij verkrijgbaar imaging softwareprogramma dat gemakkelijk door clinici gebruikt kan worden. Het gebruik van foto's maakt het mogelijk om retrospectieve analyses te verrichten, zeer kleine tot zeer grote anatomische locaties te onderzoeken, follow-up van exact dezelfde locatie te verrichten, en zelfs subtiele veranderingen in roodheid waar te nemen. Door het gebruik van de foto's is huidcontact niet nodig, waardoor de mate van roodheid van de huid niet wordt beïnvloed door veneuze compressie zoals bij een device. Wij concludeerden dat het gebruik van klinische foto's in combinatie met ImageJ® de objectieve meting van erytheem in inflammatoire ontstekingsziekten verbetert ten opzichte van observatie door het blote (klinische) oog.

In **hoofdstuk 3.4** wordt een case series beschreven van vijf patiënten die een toename vertoonden van symptomen van rosacea na gebruik van een slaapapneu (CPAP, *continuous positive airway pressure*) masker. Onze hypothese is dat het masker de huid occludeert, waardoor luchtvochtigheid, temperatuur, talggehalte, en in de darm

van *Demodex* mijten mogelijk ook bacteriële activiteit toeneemt. Dit kan aanleiding geven tot het ontstaan van rosacea, of de klachten van een reeds bestaande rosacea verergeren. Het zou interessant zijn om onze hypothese verder te onderzoeken door huidbarrière parameters objectief te bepalen bij slaapapneu-patiënten. Dit kan mogelijk leiden tot aanvullende inzichten in de mechanismes die ten grondslag liggen aan het ontwikkelen van rosacea in deze populatie.

Ten slotte werd in **hoofdstuk 3.5** systematisch de literatuur over de effectiviteit van orale bètablokkers voor rosacea-gerelateerde flushing en erytheem in het gelaat geëvalueerd. Er werden negen studies geïncludeerd. Door een grote variatie in individuele studie-uitkomsten was het lastig om deze separate resultaten met elkaar te vergelijken. Erytheem en flushing werden vastgesteld door diverse zowel subjectieve als objectieve parameters, namelijk klinische scores, laser Doppler, huidtemperatuur, en dermatoscopie. Het vergelijken van studieresultaten kan worden verbeterd door het gebruik van gestandaardiseerde en objectieve meetmethoden voor erytheem en flushing. Dit zal leiden tot meer robuuste conclusies over het effect van een specifieke behandeling.

Hoofdconclusies doelstelling 2:

- Meerdere beeldvormende en biofysische tools zijn reeds onderzocht voor het evalueren van rosacea. RCM, spectrofotometrie en bepaling van TEWL en waterinhoud lijken veelbelovend voor rosacea monitoring. Echter, grotere en gestandaardiseerde studies zijn nodig.
- Middels RCM is het mogelijk om het anti-inflammatoire effect van therapie te monitoren door *Demodex* aanwezigheid te meten, maar het tellen van exacte mijt-aantallen, inflammatoire cellen, en vasculaire parameters is uitdagend door diverse beperkingen van het RCM-apparaat. Daardoor lijkt RCM van gelimiteerde waarde voor rosacea follow-up in de dagelijkse praktijk.
- ImageJ® software maakt het mogelijk om op simpele, snelle, objectieve, en reproduceerbare wijze faciaal erytheem te kwantificeren. Het gebruik van klinische foto's maakt retrospectieve analyse, evaluatie van kleine en grote huidafwijkingen, en onderscheid van subtiele roodheidsveranderingen mogelijk.
- Het vermoeden bestaat dat slaapapneumaskers rosacea symptomen induceren, mogelijk veroorzaakt door huidocclusie, verhoging van de huidtemperatuur -en vochtigheid, en bacteriële activiteit in *Demodex* mijten. Verder onderzoek van deze hypothese kan mogelijk leiden tot inzichten in de mechanismes die rosacea veroorzaken.
- Het is lastig om individuele studie uitkomsten van behandeling van faciaal erytheem en flushing in rosacea met elkaar te vergelijken, door een grote heterogeniteit in gebruikte studie-parameters. Objectieve evaluatie van erytheem zou dit proces wel mogelijk kunnen maken.

Toekomstperspectieven

Niet-invasieve evaluatie van huidparameters biedt ons de mogelijkheid om objectieve en kwantitatieve informatie over de anatomie, functie, en eigenschappen van onze huid te verkrijgen, die niet of minder adequaat door middel van menselijke observatie verzameld had kunnen worden. Hierdoor wordt het mogelijk aan te tonen dat een ogenschijnlijk 'normale' huid toch ontstoken is, en kan een huidziekte in een eerder stadium gedetecteerd worden, nog voordat de klinische symptomen zich voordoen. Dit maakt vroege behandeling mogelijk, waarbij ernstige symptomen en irreversibele huidschade idealiter voorkomen worden. Niet-invasieve technieken hebben daarnaast het belangrijke voordeel dat de huid intact blijft en niet verstoord wordt door een invasieve meting; dit is patiëntvriendelijker (geen pijn) en voorkomt huidherstelreacties (inflammatie, verlittekening) die longitudinale metingen verstoren. In de onderzoeksetting is vergelijking van individuele studieuitkomsten eenvoudiger indien uniforme, kwantitatieve, objectieve uitkomstmaten gebruikt worden.

Vóórdat niet-invasieve technieken op grote schaal ingezet kunnen worden in de dagelijkse klinische praktijk, zijn er nog diverse technische en praktische hindernissen die overbrugd dienen te worden. Deze hindernissen worden besproken in **hoofdstuk 4**. Op basis van de resultaten en ervaringen opgedaan in dit promotieonderzoek ontwikkelden wij een lijst met karakteristieken waaraan de 'ideale tool' voor het evalueren en monitoren van inflammatoire dermatosen in de dagelijkse praktijk zou moeten voldoen. Zo zou een dergelijke tool draagbaar, lichtgewicht, zonder huidcontact en draadloos moeten zijn, in de jaszak moeten passen, of juist heel groot moeten zijn (om erin plaats te kunnen nemen, zoals een MRI). Daarnaast dient deze tool betaalbaar en commercieel beschikbaar te zijn, en de beoogde parameter snel te meten (< 5 minuten) waarbij geen herhaaldelijke kalibratie nodig is. Andere gewenste kenmerken zijn: gemakkelijk en simpel in gebruik, directe aflezing van resultaten voor directe feedback aan de patiënt, opties voor automatische retrospectieve analyse, en generatie van een overzichtsplaatje van de hele regio van interesse. Als laatste dient de tool ongevoelig te zijn voor externe factoren zoals luchtvochtigheid en temperatuur, meet het driedimensionaal tot in de subcutis, en wordt met één apparaat een kernset aan beeldvormende en biofysische parameters gecombineerd die tegelijkertijd worden gemeten.

Samenvattend, hebben wij in dit proefschrift de waarde van nieuwe en bestaande niet-invasieve technieken onderzocht in gezonde en ontstoken huid, met focus op rosacea. Wanneer de belangrijkste hindernissen overwonnen zijn, kan een wijdverspreide toepassing van deze technieken in de dermatologische praktijk verwacht worden. Wij hypothetiseren dat in de toekomst alle inflammatoire huidziekten objectief gemonitord zullen worden met de 'ideale tool', en dat dit de subjectieve scoring van de ernst van ontsteking vervangt. Idealiter leidt dit ook tot objectieve bepaling van de staat van de huid in de thuissituatie, waardoor de betrokkenheid van de patiënt bij zijn of haar ziekte, gepersonaliseerde behandeling, en therapietrouw hopelijk nog verder vergroot zullen worden.

APPENDICES

List of publications
Curriculum Vitae
Dankwoord
PhD portfolio
Research datamanagement
List of abbreviations

LIST OF PUBLICATIONS

Related to this thesis

Logger JGM, van Erp PEJ, Driessen RJB, de Jong EMGJ. The value of GPSkin to assess and monitor impaired skin barrier function. *Skin Research and Technology 2021;27:15-23*

Logger JGM, de Jong EMGJ, Driessen RJB, van Erp PEJ. Evaluation of a simple tool to quantify facial erythema in rosacea during treatment. *Skin Research and Technology 2020;26:804-812*

Logger JGM, Olydam JI, Driessen RJB. Use of beta-blockers for rosacea-associated facial erythema and flushing: a systematic review and update on proposed mode of action. *Journal of the American Academy of Dermatology 2020;83:1088-1097*

Logger JGM, Peppelman M, van Vugt R, Driessen RJB. Rosacea and use of CPAP mask for obstructive sleep apnea syndrome: report of five cases. *Annals of Dermatology 2020 Jun;32:247-250*

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Logger JGM, de Vries FMC, van Erp PEJ, de Jong EMGJ, Peppelman M, Driessen RJB. Noninvasive objective skin measurement methods for rosacea assessment: a systematic review. *British Journal of Dermatology 2020;182:55-66*

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Not related to this thesis

van den Brink NJM, Smits JPH, **Logger JGM**, Kouwenhoven TK, van Vlijmen-Willems IMJJ, Rodijk-Olthuis DRO, van den Bogaard EH. Effects of coal tar treatment after skin barrier damage in healthy volunteers. *Manuscript in preparation*

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Aarnoutse RE, Kibiki GS, Reither K, Semvua HH, Haraka F, Mtabho CM, Mpagama SG, van den Boogaard J, Sumari-de Boer IM, Magis-Escurra C, Wattenberg M, **Logger JGM**, Te Brake LHM , Hoelscher M, Gillespie SH, Colbers A, Phillips PPJ, Plemper van Balen G, Boeree MJ; PanACEA Consortium. Pharmacokinetics, Tolerability, and Bacteriological Response of Rifampin Administered at 600, 900, and 1,200 Milligrams Daily in Patients with Pulmonary Tuberculosis. *Antimicrobial Agents and Chemotherapy 2017;61:e01054-17*

CURRICULUM VITAE

Jade Gabriëlle Maria Logger werd geboren op 3 augustus 1992 in 's-Hertogenbosch en groeide op in Rosmalen. Na het cum laude behalen van haar VWO diploma aan gymnasium Bernrode te Heeswijk-Dinther in 2010, begon zij aan de bachelor biomedische wetenschappen aan de Radboud Universiteit Nijmegen, die zij in 2013 voltooide. Vervolgens gooide zij haar bloedfobie overboord en startte met de pre-master geneeskunde, gevolgd door de coschappen, waarna zij in 2017 haar artsendiploma behaalde. Aansluitend ging zij aan de slag als arts-onderzoeker/promovenda bij de afdeling Dermatologie in het Radboudumc onder begeleiding van prof. de Jong, dr. Driessen, en dr. van Erp. De resultaten van haar promotietraject staan beschreven in diverse wetenschappelijke publicaties en in dit proefschrift. Tevens heeft zij de onderzoeksresultaten gepresenteerd op verschillende congressen zoals de NVED en de Eilanddagen Dermatologie, en was zij finalist bij de wetenschappelijke pitch-wedstrijd Radboud Talks. In maart 2020 startte zij met de opleiding tot dermatoloog aan het MUMC+.

DANKWOORD

Prof. dr. E.M.G.J. de Jong, beste Elke, heel erg bedankt voor jouw begeleiding, kennis en het bewaken van de grote lijnen binnen mijn onderzoekstraject. Het was fijn dat je het grote plaatje bleef overzien en bijstuurde als de ambities iets groter bleken dan wat praktisch haalbaar is.

Dr. R.J.B. Driessen, beste Rieke, je bent direct, goudeerlijk, toegankelijk, en snel. Bij jou stond de deur altijd open, geen vraag was te dom. Wat tof dat je nu samen met Roel een publicatie hebt. Het was altijd een feestje om samen met jou de acne poli te doen. Ik heb veel bewondering voor de manier waarop je met deze kwetsbare patiënten omgaat. Dank je!

Dr. P.E.J. van Erp, beste Piet, ik heb veel geleerd van jouw onuitputtelijke biofysische kennis. Jouw enthousiasme voor het vak werkt aanstekelijk. Uit elk overleg kwamen wel één of meerdere nieuwe ideeën voor vervolgonderzoek. Je hebt me geleerd dat onderzoek een dynamisch proces is; je weet nooit precies hoe het gaat lopen. Bedankt voor jouw begeleiding en samenwerking!

Malou, bedankt voor jouw fijne begeleiding in het eerste jaar van mijn promotietraject. Je hebt me super op weg geholpen bij het opstarten van diverse onderzoeken, je hebt me de RCM leren bedienen. Jouw technische recht-door-zee blik is goud waard! *Wietske,* de 'opvolgster' van Malou; dank je wel voor de prettige samenwerking tijdens de stagebegeleiding van Jill. Jij bent echt kampioen artikelen inkorten. Heel veel succes, sterkte, en plezier verder in Friesland.

Marisol, moeder van de OIO's, PaNaMa-queen; wat zouden we toch zonder jou moeten. Ik weet niet hoe je het voor elkaar krijgt 100.000 projecten tegelijk te managen en hierbij het overzicht te bewaren. In de tussentijd ben je ook nog even gepromoveerd. Overal en altijd ben jij bereid vragen te beantwoorden, ook al ben je nog zo druk. Bedankt! *Mascha*, altijd vrolijk, geïnteresseerd en optimistisch; ik zou me geen leukere verpleegkundige kunnen voorstellen om al die RCTs mee uit te voeren dan jij! Die onesie-sessie was koning. Ik mis de chocolade!

Alle (ex-)OIO's en het 'bieb'-team; *Marieke, Finola, Marloes*, *Tamara, Elke, Lara, Mirjam, Tessa, Juul*, *Selma, Lia.* Zonder jullie was dit PhD-traject behoorlijk eenzaam geweest. Ons ommuurd fort tegenover de vergaderzaal was méér dan een vaste werkplek met twee schermen per persoon. Het was ook onze doordeweekse thuishaven om (soms iets te veel) te kletsen, frustraties af te richten, wijn te drinken, héél veel kerstversiering op te hangen, een urban jungle te creëren, en verjaardagen te vieren. Super bedankt!

Alle *lab-collegae*, ontzettend bedankt voor afgelopen jaren; jullie hulp in welke vorm dan ook, journal clubs, en gezelligheid tijdens de NVEDs. Zo fijn dat er een uitgebreid Dermatologie-lab op 5 meter loopafstand zit, en dat jullie deuren altijd open staan voor vragen (hoe moet de centrifuge aan?).

Jill en *Christina*, dank jullie wel voor jullie grote inzet en doorzettingsvermogen tijdens jullie CKO9-stage. Jullie hebben een knap staaltje onderzoek verricht met een publicatie als gevolg. Heel veel succes met jullie carrières in de Dermatologie in Rotterdam en Duitsland.

Femke, samen duizenden abstracts doorploegen met chai latte en een chocolade-brownie is zo erg nog niet. Petje af voor je geduld en wat een mooi eindresultaat hebben we bereikt. Veel succes met het afronden van jouw promotie!

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Mijn dank is groot aan alle *proefpersonen* en *patiënten* die hebben meegewerkt aan de studies. Heel erg bedankt dat u tijd heeft vrijgemaakt voor deelname. Zonder jullie waren er geen resultaten geweest. En sorry voor de tijdelijke brandmerking door het tapestrippen.

Alle *nieuwe collega's* Dermatologie van het MUMC+; dank voor jullie enthousiasme en gezelligheid op mijn nieuwe Zuid-Limburgse werkplek. *Lieke,* samen zaten wij als arts-onderzoeker in de bieb in het Radboud, en samen werden we aangenomen als aios in Maastricht. Zo leuk dat we samen dit nieuwe avontuur zijn aangegaan!

Vivienne, Esmee en *Erik,* mijn lieve vrolijke maffe vrienden. Door jullie was ik gelukkig niet 24/7 aan het werk. Tenniswedstrijden, Harry's, hardloopsessies, Oud-en-Nieuw en zomerfeesten, Malaga, Gdánsk, Roland Garros, de Intratuin; er is zoveel meer in het leven dan *P*-waardes berekenen. "Harde Kern cootjes" a.k.a. *Loes, Marieke, Sheila, Jody, Marco, Noortje, Floor, Janiek, Aafke,* en *Daniëlle*, zo leuk dat we nog regelmatig bijeen komen om spannende ziekenhuis-verhalen uit te wisselen. *Eline* en *Lara,* heerlijk om alle publicatie-struggles eruit te gooien in onze app-groep "Hart Huid Hersenen".

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Lieve *Arjen*, samen keken we uit naar het uit-eten juichmomentje als er weer een artikel geaccepteerd was, hoe hoger de impactfactor des te luxer (dus McDonalds was de max… nee grapje). Dank je voor het meelezen met de artikelen, je kritische opmerkingen, en de tijd die je me gunde om soms 's avonds of in het weekend aan mijn onderzoek te werken. Waar wonen we volgend jaar?

PHD PORTFOLIO

Name PhD candidate: J.G.M. Logger	**PhD period: 04-12-2017 to 13-11-2020**
Department: Dermatology	**Promotor: prof. E.M.G.J. de Jong**
Graduate School: Radboud Institute for Health Sciences	**Co-promotors: dr. R.J.B. Driessen and dr. P.E.J. van Erp**
Theme: inflammatory diseases	

TRAINING ACTIVITIES		
	Year(s)	ECTS
a) Courses & workshops		
• Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (BROK)	2018	1.75
• Radboud Talks (pitch presentation, *finalist)*	2018	1.75
• Patient Information Form writing	2018	1.75
• Graduate School specific introductory	2019	0.75
• Scientific Writing for PhD candidates	2019	3.0
• Project Management for PhD candidates	2019	2.0
b) Seminars & lectures		
• Seminar: DIS nascholing, Erasmus Medical Center	2018	0.1
• Radboud Research Rounds (2018: laptop presentation, 2019: laptop+oral presentation)	2018-2019	0.7
• Investigator meeting guselkumab trial, Paris	2018	0.2
• Investigator meeting LYS006 trial, München	2018	0.2
• Research Integrity round	2019	0.1
• Masterclass: acne vulgaris	2019	0.2
• Radboud Grant Round: hoe artificiële intelligentie de zorg kan verbeteren	2020	0.1
c) Symposia & congresses		
• Annual meeting Dutch Society for Experimental Dermatology, Lunteren	2018	0.5
• Farewell symposium prof. vd Kerkhof 'Imaging what is concealed: beyond Dermatology'	2018	0.25
• Annual meeting Dutch Society for Experimal Dermatology, Lunteren (poster presentation)	2019	0.75
• Farewell symposium dr. Gerritsen 'Een zonnige toekomst'	2019	0.1
• Eilanddagen Dermatologie, Schiermonnikoog (oral presentation)	2019	0.75
• Landelijke Dag NVDV, Lent (oral presentation)	2019	0.5
• Annual meeting Dutch Society for Experimental Dermatology, Lunteren (oral presentation)	2020	0.75
d) Other		
• Open door day RIMLS, stand 'Kijk op de huid'	2018	0.2
• Journal clubs (oral presentation 2 times/year)	2017-2020	3.0
• Review scientific publication (10 times)	2018-2020	1.0
e) Teaching activities		
• Supervision of two research master medical students (CKO9)	2018	2.0
• Lecture 'Demodex and Rosacea' for nurses Dermatology, Radboudumc	2018	0.1
• Supervision of Student's Meet Patient project year 1 medical students (4 times/year)	2018	0.4
• Lecture 'Rosacea and Demodex' for AIOS Dermatology, Radboudumc	2019	0.1
• Lecturing master year 5 medical students (CKO6v)	2019	0.1
Total		**22.9 ECTS**

RESEARCH DATAMANAGEMENT

This thesis is based on the results of human clinical studies, which were conducted in accordance with the principles of the Declaration of Helsinki. The medical and ethical review board Committee on Research Involving Human Subjects Region Arnhem Nijmegen, Nijmegen, the Netherlands has given approval to conduct these studies.

All projects were stored on the local Radboudumc server. Patient-related data were additionally back-upped on university servers belonging to the department (*chapter 2.3, chapter 3.3*: H:\Algemeen\Jade; *chapter 2.2, chapter 3.2:* R:\Vivascope\Backup; *chapter 2.1, chapter 2.2:* R:\Biophysica\Data). These data were not traceable to personal data; keys to personal data were stored separately.

All paperwork, including patient informed consent forms (*chapter 2.1, chapter 2.2, chapter 2.3, chapter 3.2, chapter 3.3, chapter 3.4*), patient diaries (*chapter 3.2*), and case report forms (*chapter 2.1, chapter 2.2, chapter 2.3, chapter 3.2, chapter 3.3*), were stored at the Dermatology department in a locked file cabinet (Radboudumc, room M351.00.012). Data management and monitoring was also performed digitally within Castor EDC (*chapter 2.1, chapter 2.2, chapter 3.2*) and by using password-secured Excel files (*chapter 2.3, chapter 3.3, chapter 3.4*). An audit trail was incorporated in Castor to provide evidence of the activities that altered the original data. Privacy of the participants in the studies was warranted by use of encrypted and unique individual subject numbers, not containing information regarding the identity of the patient. These numbers corresponded to the numbers on the informed consents, patient diaries, and case report forms. The keys of these numbers were stored separately from the study data at the local Radboudumc server (H:\SleutelsResearch\Sleutels\Jade). Keys were secured by a password and only accessible by the principle investigator and the study coordinator.

All data achieved will be saved for 15 years after termination of the studies. Using these patient data in future research is only possible after a renewed permission by the patient as recorded in the informed consent. Part of the results in this thesis have been published open access to make them available for everyone. The original datasets analysed during these studies are available from the associated corresponding author upon reasonable request.

LIST OF ABBREVIATIONS

AE	adverse event
AU	arbitrary units
CAIA	computer-aided image analysis
CI	confidence interval
CMOS	complementary metal-oxide semiconductor
CPAP	continuous positive airway pressure
DEJ	dermo-epidermal junction
ETR	erythematotelangiectatic rosacea
F	female
FTU	finger-tip unit
IGA	investigator's global assessment
IPL	intense pulsed light
ISD	inflammatory skin disease
KTP	potassium-titanyl-phosphate
M	male
NMF	natural moisturizing factor
OCT	optical coherence tomography
OR	odds ratio
OSAS	obstructive sleep apnea syndrome
PDL	pulsed dye laser
PPR	papulopustular rosacea
QUIPS	quality in prognosis studies
RCM	reflectance confocal microscopy
RCT	randomized controlled trial
R/G	red/green
ROI	region of interest
SC	stratum corneum
SCH	stratum corneum hydration
SG	stratum granulosum
SS	stratum spinosum
SSSB	standardized skin surface biopsy
TEWL	transepidermal water loss
TRP	transient receptor potential
UV	ultraviolet
YAG	yttrium aluminium garnet